

**COMPOSITIONS, KITS, AND METHODS FOR
IDENTIFICATION, ASSESSMENT, PREVENTION, AND THERAPY OF
PSORIASIS**

5 Related Applications

This application claims priority to U.S. Provisional Application No.: 60/203,087 filed on May 9, 2000, incorporated herein in it's entirety by this reference.

Background of the Invention

10 Psoriasis is a chronic skin disorder characterized by thickened, erythematous, well-demarcated areas of skin covered by silvery scales. The extent of involvement ranges from isolated, small lesions confined to knees, elbows, and scalp, to the whole body surface. There are several clinical forms of psoriasis, ranging from stable plaque lesions to an unstable form typified by eruptive inflammatory lesions. Psoriasis is not a
15 static disease: seasonal fluctuations, spontaneous remission, and physical and emotional well-being all affect the disease and hence its management. The disease is emotionally and physically debilitating for the subject, detracting significantly from the quality of life. Between one and three million individuals in the United States have psoriasis with nearly a quarter million new cases occurring each year. Conservative estimates place the
20 costs of psoriasis care in the United States currently at \$248 million a year.

 Psoriasis is characterized by hyper-proliferation and incomplete differentiation of epidermal keratinocytes. There are two major hypotheses concerning the pathogenesis of psoriasis. The first is that genetic factors determine abnormal proliferation of epidermal keratinocytes. The cells no longer respond normally to
25 external stimuli such as those involved in maintaining epidermal homeostasis. Abnormal expression of cell membrane cytokine receptors or abnormal transmembrane signal transduction might underlie cell hyperproliferation. Inflammation associated with psoriasis is secondary to the release of pro-inflammatory molecules from hyperproliferative keratinocytes.

30 A second hypothesis is that T cells interacting with antigen-presenting cells in skin release pro-inflammatory and keratinocyte-stimulating cytokines (Hancock, G. E. et al., J. Exp. Med. 168:1395-1402, 1988). Only T cells of genetically predetermined individuals possess the capacity to be activated under such circumstances. The keratinocytes themselves may be the antigen-presenting cell. The cellular infiltrate in
35 psoriatic lesions show an influx of CD4+ T cells and, more prominently, CD8.sup.+ T

cells (Bos, J. D. *et al.*, *Arch. Dermatol. Res.* 281:23-3, 1989; Baker, B. S., *Br. J Dermatol.* 110:555-564, 1984).

Keratinocyte hyperplasia in psoriasis has been linked to overproduction of cytokines such as TGF α and interleukin-6 (IL-6) and overexpression of epidermal growth factor receptor (EGF-R) in affected skin (Krueger, *et al.*, *J. Invest. Dermatol.*, 94:1355-1405, 1990). EGF-R is a 180-kD cell-surface receptor whose activity is regulated by both EGF and TGF α . In psoriasis vulgaris, EGF-R persists throughout the epidermis from the basal layers to the stratum corneum. Such persistent EGF-R has been shown to be biologically active *in vivo* in nude mice (Nanney, *et al.*, *J. Invest. Dermatol.*, 98:296-301, 1992).

Various studies have also shown increased enzyme activity in polymorphonuclear (PMN) leukocytes in the circulation of subjects with psoriasis. Open and co-workers (Clin. Chine. ACTA 264:49, 1997) in Turkey correlated elastase levels in PMN leukocytes to disease activity. The PMN elastase levels were six-fold higher in psoriatic subjects with active disease compared to controls, but only two-fold higher during quiescent stages of psoriasis. Not only is the elastase a sensitive marker of disease activity, it is also reflective of a concomitant inflammatory reaction which gives rise to free radical species. Elastase levels in PMNs also correlated with the total white blood cell (PMN) count and the levels of another reflector of inflammation, alpha-1-antitrypsin.

There are various types of psoriasis and thus there are a number of effective treatments or combinations, but not one that is specific or curative (see Stiller, M. J., A Management Update on Psoriasis, Hospital Medicine, January 1994, pp. 28-35). Therapeutic treatments include the use of corticosteroids, coal tar, bath solutions (*e.g.*, salts), retinoids, vitamin D3, occlusion therapy, cyclosporine and methotrexate, among others. Psoralens have been used in combination with ultraviolet A radiation, despite its known carcinogenic properties. Ultraviolet B radiation has also been used successfully in some cases. U.S. Pat. Nos. 4,513,011; 4,495,203; 4,507,321; 4,933,330; 4,847,257; 4,496,588; 4,981,681; and 4,518,789, the contents of which are incorporated herein by reference, disclose compositions for the treatment of psoriasis. See also U.S. FDA OTC Drug Monograph, Federal Register, Vol. 47, No. 233 (Dec. 3, 1982) pp. 54646-54684, the contents of which are incorporated by reference, which lists OTC compositions for treatment of psoriasis. However, many topical products currently available are irritating, messy or simply ineffective. Topical steroids account for 90% of the psoriasis market in the United States and have many side effects including cutaneous atrophy, telangiectasia, formation of striae and tachyphylaxis.

Summary of the Invention

- In one embodiment, the invention provides a method of assessing whether a subject is afflicted with psoriasis or a TH1-associated condition, by comparing the level of expression of a marker in a sample from a subject, where the marker is selected from the group of markers set forth in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7 to the normal level of expression of the marker in a control sample, where a significant difference between the level of expression of the marker in the sample from the subject and the normal level is an indication that the subject is afflicted with psoriasis or a TH1-associated condition.
- 5 In a preferred embodiment, the marker corresponds to a transcribed polynucleotide or portion thereof, where the polynucleotide includes the marker. In a particularly preferred embodiment, the level of expression of the marker in the sample differs from the normal level of expression of the marker in a subject not afflicted with psoriasis or a TH1-associated condition by a factor of at least two, and in an even more preferred
- 10 embodiment, the expression levels differ by a factor of at least five. In another preferred embodiment, the marker is not significantly expressed in noninvolved tissue.
- In another preferred embodiment, the sample includes cells obtained from the subject. In another preferred embodiment, the level of expression of the marker in the sample is assessed by detecting the presence in the sample of a protein corresponding to
- 15 the marker. In a particularly preferred embodiment, the presence of the protein is detected using a reagent which specifically binds with the protein. In an even more preferred embodiment, the reagent is selected from the group of reagents including an antibody, an antibody derivative, and an antibody fragment. In another preferred embodiment, the level of expression of the marker in the sample is assessed by detecting
- 20 the presence in the sample of a transcribed polynucleotide or portion thereof, where the transcribed polynucleotide includes the marker. In a particularly preferred embodiment, the transcribed polynucleotide is an mRNA or a cDNA. In another particularly preferred embodiment, the step of detecting further comprises amplifying the transcribed polynucleotide.
- 25 In yet another preferred embodiment, the level of expression of the marker in the sample is assessed by detecting the presence in the sample of a transcribed polynucleotide which anneals with the marker or anneals with a portion of a polynucleotide under stringent hybridization conditions, where the polynucleotide includes the marker. In another preferred embodiment, the level of expression in the
- 30 sample of each of a plurality of markers independently selected from the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7 is compared with the normal level of expression of each of
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the plurality of markers in samples of the same type obtained from control subjects not afflicted with psoriasis or a TH1-associated condition, where the level of expression of more than one of the markers is significantly altered, relative to the corresponding normal levels of expression of the markers, is an indication that the subject is afflicted with psoriasis or a TH1-associated condition. In a particularly preferred embodiment, the plurality includes two or more of the markers. In a still more preferred embodiment, the plurality includes at least five of the markers set forth in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7.

In another embodiment, the invention provides a method for monitoring the progression of psoriasis or a TH1-associated condition in a subject, including detecting in a subject sample at a first point in time the expression of marker, where the marker is selected from the group including the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, repeating this detection step at a subsequent point in time, and comparing the level of expression detected in the two detection steps, and monitoring the progression of psoriasis or a TH1-associated condition in the subject using this information. In a preferred embodiment, the marker is selected from the group including the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7 and combinations thereof. In another preferred embodiment, the marker corresponds to a transcribed polynucleotide or portion thereof, where the polynucleotide includes the marker. In another preferred embodiment, the sample includes cells obtained from the subject. In a particularly preferred embodiment, the cells are collected from skin or blood tissue.

In another embodiment, the invention provides a method of assessing the efficacy of a test compound for inhibiting psoriasis or a TH1-associated condition in a subject, including comparing expression of a marker in a first sample obtained from the subject which is exposed to or maintained in the presence of the test compound, where the marker is selected from the group including the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, to expression of the marker in a second sample obtained from the subject, where the second sample is not exposed to the test compound, where a significantly lower level of expression of the marker in the first sample relative to that in the second sample is an indication that the test compound is efficacious for inhibiting psoriasis or a TH1-associated condition in the subject. In a preferred embodiment, the first and second samples are portions of a single sample obtained from the subject. In another preferred embodiment, the first and second samples are portions of pooled samples obtained from the subject.

In another embodiment, the invention provides a method of assessing the efficacy of a therapy for inhibiting psoriasis or a TH1-associated condition in a subject, the method including comparing expression of a marker in the first sample obtained from the subject prior to providing at least a portion of the therapy to the subject, where
5 the marker is selected from the group including the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, to expression of the marker in a second sample obtained from the subject following provision of the portion of the therapy, where a significantly lower level of
10 expression of the marker in the second sample relative to the first sample is an indication that the therapy is efficacious for inhibiting psoriasis or a TH1-associated condition in the subject.

In another embodiment, the invention provides a method of assessing the efficacy of a therapy for inhibiting psoriasis or a TH1-associated condition in a subject, the method including comparing expression of a marker in the first sample obtained
15 from the subject prior to providing at least a portion of the therapy to the subject, where the marker is selected from the group including the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, to expression of the marker in a second sample obtained from the subject following provision of the portion of the therapy, where a significantly enhanced level of
20 expression of the marker in the second sample relative to the first sample is an indication that the therapy is efficacious for inhibiting psoriasis or a TH1-associated condition in the subject.

In another embodiment, the invention provides a method of selecting a composition for inhibiting psoriasis or a TH1-associated condition in a subject, the
25 method including obtaining a sample including cells from a subject, separately maintaining aliquots of the sample in the presence of a plurality of test compositions, comparing expression of a marker in each of the aliquots, where the marker is selected from the group including the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, and selecting one
30 of the test compositions which induces a lower level of expression of the marker in the aliquot containing that test composition, relative to other test compositions.

In another embodiment, the invention provides a method of selecting a composition for inhibiting psoriasis or a TH1-associated condition in a subject, the method including obtaining a sample including cells from a subject, separately
35 maintaining aliquots of the sample in the presence of a plurality of test compositions, comparing expression of a marker in each of the aliquots, where the marker is selected from the group including the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A,

4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, and selecting one of the test compositions which induces an enhanced level of expression of the marker in the aliquot containing that test composition, relative to other test compositions.

5 In another embodiment, the invention provides a method of inhibiting psoriasis or a TH1-associated condition in a subject, including obtaining a sample including cells from a subject, separately maintaining aliquots of the sample in the presence of a plurality of test compositions, comparing expression of a marker in each of the aliquots, where the marker is selected from the group including the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6
10 Group IV, and 7, and administering to the subject at least one of the test compositions which induces a lower level of expression of the marker in the aliquot containing that test composition, relative to other test compositions.

In another embodiment, the invention provides a method of inhibiting psoriasis or a TH1-associated condition in a subject, including obtaining a sample including cells
15 from a subject, separately maintaining aliquots of the sample in the presence of a plurality of test compositions, comparing expression of a marker in each of the aliquots, where the marker is selected from the group including the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, and administering to the subject at least one of the test compositions
20 which induces a higher level of expression of the marker in the aliquot containing that test composition, relative to other test compositions.

In another embodiment, the invention provides a kit for assessing whether a subject is afflicted with psoriasis or a TH1-associated condition, including reagents for assessing expression of a marker selected from the group including the markers listed in
25 Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7.

In another embodiment, the invention provides a kit for assessing the presence of psoriatic cells or cells participating in a TH1-associated condition, the kit including a nucleic acid probe where the probe specifically binds with a transcribed polynucleotide
30 corresponding to a marker selected from the group including the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7.

In another embodiment, the invention provides a kit for assessing the suitability of each of a plurality of compounds for inhibiting psoriasis or a TH1-associated
35 condition in a subject, the kit including a plurality of compounds and a reagent for assessing expression of a marker selected from the group including the markers listed in

In another embodiment, the invention provides a kit for assessing the presence of psoriatic cells or cells participating in a TH1-associated condition, including an antibody, where the antibody specifically binds with a protein corresponding to a marker selected from the group including the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7.

In another embodiment, the invention provides a kit for assessing the presence of psoriatic cells or cells participating in a TH1-associated condition, the kit including a nucleic acid probe where the probe specifically binds with a transcribed polynucleotide corresponding to a marker selected from the group including the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7.

In another embodiment, the invention provides a method of assessing the potential of a test compound to trigger psoriasis or a TH1-associated condition in a cell, including maintaining separate aliquots of cells in the presence and absence of the test compound, and comparing expression of a marker in each of the aliquots, where the marker is selected from the group including the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, where a significantly enhanced level of expression of the marker in the aliquot maintained in the presence of the test compound, relative to the aliquot maintained in the absence of the test compound, is an indication that the test compound possesses the potential for triggering psoriasis or a TH1-associated condition in a cell.

In another embodiment, the invention provides a method of assessing the potential of a test compound to trigger psoriasis or a TH1-associated condition in a cell, including maintaining separate aliquots of cells in the presence and absence of the test compound, and comparing expression of a marker in each of the aliquots, where the marker is selected from the group including the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, where a significantly decreased level of expression of the marker in the aliquot maintained in the presence of the test compound, relative to the aliquot maintained in the absence of the test compound, is an indication that the test compound possesses the potential for triggering psoriasis or a TH1-associated condition in a cell.

In another embodiment, the invention provides a kit for assessing the potential
35 for triggering psoriasis or a TH1-associated condition in a cell of a test compound,
including cells and a reagent for assessing expression of a marker, where the marker is

selected from the group including the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7.

5 In another embodiment, the invention provides a method of treating a subject afflicted with psoriasis or a TH1-associated condition, including providing to cells of the subject afflicted with psoriasis or a TH1-associated condition a protein corresponding to a marker selected from the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7. In a preferred embodiment, the protein is provided to the cells by providing a vector including a polynucleotide encoding the protein to the cells.

10 In another embodiment, the invention provides a method of treating a subject afflicted with psoriasis or a TH1-associated condition an antisense oligonucleotide complementary to a polynucleotide corresponding to a marker selected from the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7.

15 In another embodiment, the invention provides a method of inhibiting psoriasis or a TH1-associated condition in a subject at risk for developing psoriasis or a TH1-associated condition, including inhibiting expression of a gene corresponding to a marker selected from the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7.

20 In another embodiment, the invention provides a method of inhibiting psoriasis or a TH1-associated condition in a subject at risk for developing psoriasis or a TH1-associated condition, the method comprising enhancing expression of a gene corresponding to a marker selected from the markers listed in Tables 1-8, 10, and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and
25 7.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

30 **Brief Description of the Drawings**

Figure 1 depicts a table of marker protein expression in skin samples from patients undergoing cyclosporin A treatment for psoriasis, as compared to control, uninvolved (*e.g.*, nonpsoriatic) skin samples. Lightly shaded values indicate a marker expression level that is at least two-fold decreased from control values. Darkly shaded values indicate a marker expression level that is at least two-fold increased from control values.

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Figure 2 depicts a table of marker protein expression in skin samples from patients undergoing recombinant human IL-11 (rhIL-11) treatment for psoriasis, as compared to control, uninvolved (e.g., nonpsoriatic) skin samples. Lightly shaded values indicate a marker expression level that is at least two-fold decreased from control values.

- 5 Darkly shaded values indicate a marker expression level that is at least two-fold increased from control values.

Figure 3 depicts a table describing 165 statistically significant differentially expressed genes between uninvolved and involved psoriasis skin. Uninvolved and involved skin from 6 psoriasis patients was obtained and RNA hybridized to
10 oligonucleotide arrays. Average frequency values and standard deviations of 6 uninvolved samples and 6 lesional samples were calculated. A paired students t test was performed and p values are presented. Values below 0.05 were considered statistically significant. Fold change in gene expression was calculated as the ratio of the average frequency of lesional skin compared to uninvolved skin. Data is presented as fold
15 change and is coded according to the average frequency value. Genes are grouped according to functional significance.

Figures 4A-4B depict graphs of RT-PCR confirmation of some chip results in larger patient populations. (A) Genes that are elevated in psoriatic lesions versus uninvolved skin. (B) Genes that are elevated in uninvolved skin versus psoriatic lesions.
20 Uninvolved and lesional skin from 24 psoriasis patients was analyzed in pairwise fashion. Average expression values and standard deviations for uninvolved and lesional skin was calculated. Student's t test was performed and statistical significance is indicated.

Figures 5A-5D depict the following. (A) Ven diagram comparison of DTH, tape
25 strip, psoriasis gene expression results. Genes differentially regulated by 2-fold or greater between uninvolved and lesional psoriasis skin, between normal and DTH skin and between normal and tape stripped skin was calculated. Overlap between the various sample types was calculated. (B) Histograms of 29 genes differentially regulated following tape stripping and in psoriasis lesions. (C) Histograms of 26 genes
30 differentially regulated following a DTH reaction and in psoriasis lesions. (D). Histograms of 16 genes differentially regulated following a DTH reaction, tape stripping and in psoriasis lesions.

Figure 6 depicts SOM Analysis of selected genes following IL-11 and cyclosporin treatments. Oligonucleotide arrays were performed on uninvolved and
35 lesional skin from 7 patients treated with rhIL-11 or cyclosporin A. Biopsies were obtained at baseline, week 1, week 4, week 8 and week 12 of treatment. Patients were grouped into responder (n=4) and non-responder (n=3) categories based on clinical and

histopathological criteria. Average expression values in lesional skin at baseline, week 1, week 4, week 8 and week 12 for responder and non-responder groups were calculated. A 4 cluster (1 X 4) SOM was generated using the 28 biopsy samples. Genes contained in each SOM are shown to the right of the maps. Genes are coded according to

5 psoriasis disease gene loci.

Figure 7 depicts graphs of early response gene analysis in larger patient populations by RT-PCR. Skin biopsies were obtained from 24 patients treated with rhIL-11 or cyclosporin A at baseline, week 1, week 4, week 8, and week 12 of therapy. Patients were divided into treatment responders and non-responders based on a

10 combination of clinical and histopathological criteria. Quantitative RT-PCR was performed on selected genes. Average TaqMan units and standard deviations for patients at each time point were calculated and are presented. Statistically significant differences ($p < 0.05$) are represented by *.

15 **Detailed Description of the Invention**

The invention relates, in part, to newly discovered correlations between the expression of selected markers and the presence of psoriasis or a TH1-associated condition in a subject. The relative levels of expression of these markers, both alone and in combination, have been found to be indicative of a predisposition in the subject to

20 psoriasis and/or diagnostic of the presence or potential presence of psoriasis in a subject. The invention provides panels of markers, methods for detecting the presence or absence of psoriasis or a TH1-associated condition in a sample or subject, and methods of predicting the incidence of psoriasis or a TH1-associated condition in a sample or subject. The invention also provides methods by which psoriasis or a TH1-associated

25 condition may be treated, using the markers of the invention.

The present invention is based, at least in part, on the identification of a number of genetic markers, set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, which are differentially expressed in samples from skin tissue involved in psoriatic lesions than they are in normal,

30 uninvolved skin tissue. A panel of 6800 known genes was screened for expression in psoriatic versus non-psoriatic tissue from four different subjects afflicted with the disease (see Example 1). Those genes with statistically significant ($p < .05$) differences between the diseased and normal tissues are identified. This differential expression was observed either as a decrease in expression (e.g., Table 1), or an increase in expression

35 (e.g., Table 2).

In addition, using polymerase chain reaction (PCR) analyses, which are capable of detecting expression of a single transcript in a population of 10^6 nucleic acids, several

additional genes which were differentially expressed in psoriasis, but which, due to the sample size, had not been deemed in the previous analysis to be statistically significant, were identified. The expression of these selected genes in skin samples from involved and non-involved tissue from three different psoriasis-afflicted subjects was assessed by
5 GeneChip analysis, as described in Example 1 (Table 8). Defensin, PD-ECGF, TPA, MMP-12, S100A8, S100A2, IFI-27, MMP-16, and MMP-2 were found to increase in expression in psoriatic tissue as compared to noninvolved tissue, whereas the reverse was observed for TIMP-3, APO-E, ID4, and PBX2 (Table 8).

Included among the genes on the panel used to screen psoriatic versus non-
10 psoriatic tissue as an internal control were four genes known in the art to be implicated in psoriasis, set forth in Table 9. As is shown in Table 2, each of these four genes were found to be significantly increased in expression in psoriatic skin cells as opposed to nonpsoriatic skin cells. Several of the other identified marker genes in Tables 1 and 2 were found to map to three chromosomal loci which were previously (Balendran *et al. J.*
15 *Invest. Derm.* 113: 322-328, 1999; Nair *et al. Hum. Molec. Genet.* 6: 1349-1356, 1997; and Capon *et al. J. Hum. Genet.* 65: 1798-1800, 1999) found to be associated with psoriasis in linkage studies: 6p21.3, 17q, and 1q21 (see Tables 6 and 7). While the genes in Tables 6 and 7 are known in the art, they have not previously been associated with psoriasis or any TH1-associated condition.

20 Blood samples from subjects afflicted with psoriasis were also analyzed (Table 10) (Example 2A). Some overlap in genes with differential regulation was observed between the blood and skin cell results, although certain of the genes were unique to each tissue (Tables 1, 2, 8, and 10).

To differentiate genes with altered expression in psoriasis from genes with
25 altered expression in general inflammation reactions, expression of the genes which were identified (as described herein) as being over- or under-expressed in psoriatic tissue was assessed in tissue undergoing a nonpsoriatic immune or inflammatory reaction (see Example 2B). Localized inflammatory responses were induced by the application and rapid removal of tape from the nonpsoriatic skin of a subject; gene expression in skin
30 cells taken from this inflamed region was assessed (Tables 1-5). Alternatively, cells from an unaffected subject or from noninvolved skin tissue were exposed to an antigen, in order to generate a delayed type hypersensitivity (DTH) reaction. This reaction is known to manifest in induration, swelling and monocytic infiltration into the site of the lesion within 24 to 72 hours. Gene expression in this inflamed tissue was assessed as
35 above (Tables 1-5). As a result, genes whose expression is associated with psoriasis are set forth in Table 3, and further include the STAT3 and TUBB2 genes set forth in Table

5, whereas genes which are differentially expressed both in response to psoriasis and also to general inflammatory or immune reactions are set forth in Tables 1 and 4.

While the etiology of psoriasis remains unknown, the activity of T-helper-1 (TH1) cells is believed to play a role in the origin and/or progression of this disease, as well as multiple sclerosis, Crohn's disease, and rheumatoid arthritis. Accordingly, the differential expression of the genes identified as described herein in activated T-helper cells was assessed. Th1 and Th2 cells were exposed to the phorbol ester PMA and/or to ionomycin, thereby activating these cells, and gene expression was assessed (see Example 2C). As is shown in Table 1, a number of the genes are increased in expression in activated Th1 cells over activated Th2 cells.

To identify a psoriasis gene classification set, a differential mRNA expression profile of non-lesional to lesional skin from the subset of 8 patients with psoriasis was performed. A large number of differentially regulated genes were identified. 340 to 1321 genes were found to increase or decrease by an average factor of 2-fold or greater within the individual patients (Table 11). Comparison of normal skin to uninvolved psoriasis skin also indicated a high degree of similarity with only 34 genes differing by 2-fold or greater. 476 genes were identified that were statistically significantly different between these two defined groups with a confidence level of 95% or greater. This list was further refined to select only those genes whose expression levels differed on average by 2-fold or greater between non-lesional and lesional skin. 159 genes fit this second criteria (Figure 3). Class prediction analysis using metrics defined by Golub *et al.* ((1999) *Science* 286:531-537) indicated that this 159 gene set can be used to predict with 100% accuracy expression patterns unique to normal, non-lesional or lesional skin. These 159 genes therefore comprise a disease classification set for plaque psoriasis.

Within this set, many genes not previously associated with psoriasis were also identified as being differentially regulated. For example, overexpression of multiple S100 family members such as S100A12 (calgizarin C, ENRAGE), S100A11 and S100A2, matrix metalloproteinases (MMP-12) and heparin binding protein 17 (HBP-17) were observed (Figure 3). MMP-12 has previously been shown through microarray analysis to be deregulated in Crohn's and RA tissue, and HBP17, has been shown to play a role in angiogenesis and wound healing. A number of genes such as keratin 2, Apolipoprotein E (APOE), GATA3, Rb1, calponin 1 (CNN1), Cystatin 6 (CST6), TIMP-3 and TNXA were downregulated in the psoriatic lesions versus non-lesional skin. Other genes involved in inflammation and immune regulation such as the IL-4R, CD2, CD24, CD47, STAT-1, IFI27, IFI56, MX1, MnSOD, and MCP1 were elevated in lesional versus non-lesional tissue (Figure 3).

Analysis of 16 additional psoriasis patients confirmed elevation of HARP, S100A12, HBP17, IL-4R, CCNF, LAD1, MAPKK3b, MMP-12, MTX, and DSG3 mRNA levels in lesional skin (Figure 4A). Lower levels of CST6, TNXA, ID4, TIMP-3, GATA-3, IL-5, and ApoE in lesional skin versus non-lesional skin were also confirmed (Figure 4B).

Expression profiles were generated for other cutaneous inflammatory conditions (e.g., delayed type hypersensitivity reaction (DTH) and tape stripping) with different mechanistic components to further characterize the role of these differentially regulated genes in the pathophysiology of psoriasis. A comparison of a biopsy obtained at the site of the DTH reaction to a noninvolved region identified from 182 to 925 genes that were differentially regulated between normal and lesional skin (Table 11). On average, 259 genes differed by 2-fold or greater in all subjects tested (Figure 5A). Biopsies were also obtained from normal skin and tape stripped skin from three different subjects. Anywhere from 62 to 309 genes differed in normal versus lesional skin across the 3 volunteers (Table 11). 161 genes were identified that were differentially regulated on average 2-fold or greater in all 3 tape stripped samples (Figure 5A).

A comparison of these expression profiles to the 159 psoriasis disease genes identified herein was performed (Figure 5A). Genes that were differentially regulated in a qualitative manner were grouped together. 13 genes were found to be differentially regulated in all three inflammatory states: SCCA2, KRT16, KRT6E, PSRR1B, GNA15, MX1, SRM, ZPF36, S100A2, S100A7, S100A9, PI3 and DEFB2 (Figure 5B). 94 genes including S100A12 (ENRAGE), RAGE and GATA-3 were specifically regulated only in psoriasis. 23 genes were differentially regulated in psoriasis and following a DTH reaction: PSMB10, SCAA1, IFI27EP, PSMA28, CD47, SCYA2, PRSS6, IL4R, PLSCR1, CD2, CHIT1, SOD2, CDC25, CASP4, 3PK, LGALS3BP, THBD, H2AZ, HAL, ARG1, GARS, ALDH10, and ATP1B1 (Figure 5C). 30 genes were differentially regulated in psoriasis and following tape stripping: KRT17, HBP17, CTSL, PRSS3, SPRR2A, CD24, HSPA8, LAD1, EIF5A, UP, ANT2, BENE, K98_TCP1, RANBP1, PCBD, MDF1, SF2P32, DSG3, TOP1, DDX, K5, PHB, PCSK4, CEBPD, ELP1, ETR101, LDLR, EIF5, KRT2A, and OSF2OS (Figure 5D). 11 genes were in common between DTH and tape stripped but not psoriasis.

Multiple differentially expressed genes mapped to the 6 identified psoriatic susceptibility loci (Table 12). For example, RAGE, MDF1, ID4 and TNXA mapped to the *PSOR1* loci on 6p21.3. MDF1 was overexpressed in lesional tissue and ID4, RAGE and TNXA were downregulated in lesional versus non-lesional tissue. ID4, located in the HLA locus on 6p21.3, is a dominant/negative regulator of the basic helix-loop-helix (bHLH) family of transcription factors and serve as general antagonists of cellular

differentiation and proliferation in a variety of cell lineages (Riechmann *et al.* (1994) *Nuc. Acids Res.* 22:749-755; Pagliuca *et al.* (1995) *Genomics* 27:200-203). Cellular injury results in a downregulation of ID4 leading to a reduction in apoptosis (Andres-Barquin *et al.* (1998) *Neuroreport* 9:4075-4080; Andres-Barquin *et al.* (1999) *Exp. Cell Res.* 247:347-355). Reduction of ID4 expression in the epidermis, through either inherited mutations or following insult, may lead to deregulation of a number of downstream bHLH transcriptions factors that control keratinocyte growth.

Differential expression of several genes at the PSOR2 loci on chromosome 17q including a member of the MAP kinase family (PRKMK3; MEK3), and two genes involved in inflammatory processes, the chemokine SCYA2 and the Mac-2 binding protein, were observed. PRKMK3 is mitogen-activated protein kinase kinase 3 (MKK3), an upstream regulator of p38 MAPK (Enslen *et al.* (1998) *J. Biol. Chem.* 273:1741-1748). p38 MAPK is activated by proinflammatory molecules such as TNF- α and plays a role in the inflammatory process. Blocking p38 activity results in a reduction in the inflammatory state (Herlaar *et al.* (1999) *Mol. Med. Today* 5:439-447).

Several genes that map to the PSOR4 loci on chromosome 1q21 were also overexpressed in lesional tissue such as MTX and multiple members of the S100 gene family such as S100A12, S100A2 and S100A9. Interestingly, S100A12 (calgranulin C) is a ligand for the RAGE receptor that mapped to the PSOR1 loci at 6p21.3 (Hofman *et al.* (1999) *Cell* 97:889-901). S100A12, located on 1q21, has been implicated in activation of the proinflammatory NF- κ B pathway. One gene, ACPP, mapped to the PSOR5 loci on 3q21 and three genes involved in signal transduction (CNN1, GNA15) or LDL signaling (LDLR) mapped to the PSOR6 loci on 19p13. Quantitative RT-PCR analysis for a selected group of these genes, such as PRKMK3, HBP17, S100A12, MTX, TNXA and ID4, in a larger patient population confirmed the initial gene chip findings and indicated concordance for this deregulation in all patients examined (Figure 4A and B).

To identify genes whose expression patterns change over the course of drug treatment, comparisons in expression profiles of the lesional skin was made between responding and non-responding subjects prior to and during the course of drug treatment. Self-organizing maps (SOMs) were employed to aid in the identification of pharmacogenomic expression patterns (Tamayo *et al.* (1999) *Proc. Nat. Acad. Sci.* 96:2907-2912). Subjects were treated with either rhIL-11 or cyclosporin A. Four patterns of gene expression that change over the course of drug treatment in responding patients were identified in this gene set (Figure 6). In contrast, non-responding patients saw no significant change in expression patterns of this gene set over the course of drug treatment.

It is believed that changes in gene expression that precede clinical improvement may play a more causal role in diseases progression as opposed to genes whose expression changes mirror clinical improvement or do not change despite clinical improvement. This approach identified a subset of 36 differentially regulated genes that returned to normal or uninvolved levels at time points that preceded clinical improvement following therapeutic intervention with rhIL-11 or cyclosporin A (Figure 6, Group I). Members of this group included genes such as ID4, HBP-17, KRT16, S100A2, S100A9, S100A12, GNA15, MTX, PRKMK3, and SCYA2 that all localized to psoriasis disease loci. In addition, a number of immune modulatory genes such as IL-4R, CD2 and GATA3 fell into this category.

The second pattern corresponded to genes whose levels returned to those of non-lesional skin but only at the end of drug therapy and not prior to clinical improvement (Figure 6, Group II). This cluster included 97 genes out of the 165 genes that were differentially regulated. The third expression pattern included 19 genes whose expression levels did not change over the course of therapy despite clinical improvement in the patients skin lesions (Figure 6, group III). The final cluster contained genes whose expression levels increased over the course of drug treatment. Changes in the expression levels of some of these genes such as TNXA, RAGE, ID4 and GATA-3 also proceeded clinical improvement (Figure 6, Group IV).

Changes in gene expression levels that precede clinical improvement may identify genes that play a potential causative role in disease progression or may be early markers of clinical efficacy. Quantitative RT-PCR was used to analyze gene expression changes for these early responding genes in a larger patient population. Expression levels of genes for S100A12, HBP17, K16, CCNF, SCYA2, PRKMK3, ID4, CST6 and TNXA were analyzed in 15 patients treated with rhIL-11 and 9 patients treated with cyclosporin A (Figure 7). Consistent with the above analysis, similar changes in gene expression in response to drug treatment were observed as early as 1 week following drug therapy in responding patients but not in non-responding patients. In most instances, this change in gene expression preceded clinical improvement in these patients. Following discontinuation of drug treatment, mRNA expression levels for some of these genes such as K16, S100A12 and CCNF began to rebound at week 12 towards those found in untreated lesions further supporting a causative role for these genes in disease progression (Figure 7). These results also indicate that these genes or pathways defined by them may be appropriate therapeutic intervention points.

Accordingly, the present invention pertains to the use of the genes set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7 (e.g., the DNA or cDNA) or subset thereof, the corresponding

mRNA transcripts, and the encoded polypeptides as markers for the presence or risk of development of psoriasis or a TH1-associated condition. These markers are further useful to correlate the extent and/or severity of disease. Panels of the markers can be conveniently arrayed for use in kits or on solid supports. The markers can also be useful
5 in the treatment of psoriasis or a TH1-associated condition, or in assessing the efficacy of a treatment for psoriasis or a TH1-associated condition.

In one aspect, the invention provides markers whose quantity or activity is correlated with the presence of psoriasis or a TH1-associated condition. The markers of the invention may be nucleic acid molecules (*e.g.*, DNA, cDNA, or RNA) or
10 polypeptides. These markers are either increased or decreased in quantity or activity in psoriatic tissue as compared to nonpsoriatic tissue. For example, the gene designated 'BENE' (accession number U17077) is increased in expression level in psoriatic tissue (Table 3), while the gene designated 'CRIP1' is decreased in expression levels in psoriatic tissue relative to nonpsoriatic tissue (Table 1). Both the presence of increased
15 or decreased mRNA for these genes (and for other genes set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7), and also increased or decreased levels of the protein products of these genes (and other genes set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7) serve as markers of psoriasis or a TH1-
20 associated condition. Preferably, increased and decreased levels of the markers of the invention are increases and decreases of a magnitude that is statistically significant as compared to appropriate control samples (*e.g.*, samples not affected with psoriasis or a TH1-associated condition). In particularly preferred embodiments, the marker is increased or decreased relative to control samples by at least 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-,
25 or 10-fold or more. Similarly, one skilled in the art will be cognizant of the fact that a preferred detection methodology is one in which the resulting detection values are above the minimum detection limit of the methodology.

Measurement of the relative amount of an RNA or protein marker of the invention may be by any method known in the art (see, *e.g.*, Sambrook, J., Fritsh, E. F.,
30 and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Typical methodologies for RNA detection include RNA extraction from a cell or tissue sample, followed by hybridization of a labeled probe (*e.g.*, a
35 complementary nucleic acid molecule) specific for the target RNA to the extracted RNA, and detection of the probe (*e.g.*, Northern blotting). Typical methodologies for protein detection include protein extraction from a cell or tissue sample, followed by

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hybridization of a labeled probe (*e.g.*, an antibody) specific for the target protein to the protein sample, and detection of the probe. The label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Detection of specific protein and nucleic acid molecules may also be assessed by gel electrophoresis, column
5 chromatography, direct sequencing, or quantitative PCR (in the case of nucleic acid molecules) among many other techniques well known to those skilled in the art.

In certain embodiments, the genes themselves (*e.g.*, the DNA or cDNA) may serve as markers for psoriasis or a TH1-associated condition. For example, the absence of nucleic acids corresponding to a gene (*e.g.*, a gene from Table 1), such as by deletion
10 of all or part of the gene, may be correlated with disease. Similarly, an increase of nucleic acid corresponding to a gene (*e.g.*, a gene from Table 2), such as by duplication of the gene, may also be correlated with disease.

Detection of the presence or number of copies of all or a part of a marker gene of the invention may be performed using any method known in the art. Typically, it is
15 convenient to assess the presence and/or quantity of a DNA or cDNA by Southern analysis, in which total DNA from a cell or tissue sample is extracted, is hybridized with a labeled probe (*e.g.*, a complementary DNA molecule), and the probe is detected. The label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Other useful methods of DNA detection and/or quantification include direct
20 sequencing, gel electrophoresis, column chromatography, and quantitative PCR, as is known by one skilled in the art.

The invention also encompasses nucleic acid and protein molecules which are structurally different from the molecules described above (*e.g.*, which have a slightly altered nucleic acid or amino acid sequence), but which have the same properties as the
25 molecules above (*e.g.*, encoded amino acid sequence, or which are changed only in nonessential amino acid residues). Such molecules include allelic variants, and are described in greater detail in subsection I.

In another aspect, the invention provides markers whose quantity or activity is correlated with the severity of psoriasis or a TH1-associated condition (see, *e.g.*,
30 Example 3). These markers are either increased or decreased in quantity or activity in psoriatic tissue in a fashion that is either positively or negatively correlated with the degree of severity of the psoriasis or TH1-associated condition. In yet another aspect, the invention provides markers whose quantity or activity is correlated with a risk in a subject for developing psoriasis or a TH1-associated condition. These markers are either
35 increased or decreased in activity or quantity in direct correlation to the likelihood of the development of psoriasis or a TH1-associated condition in a subject.

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Each marker may be considered individually, although it is within the scope of the invention to provide combinations of two or more markers for use in the methods and compositions of the invention to increase the confidence of the analysis. In another aspect, the invention provides panels of the markers of the invention. In a preferred embodiment, these panels of markers are selected such that the markers within any one panel share certain features. For example, the markers of a first panel may each exhibit an increase in quantity or activity in psoriatic tissue as compared to non-psoriatic tissue, whereas the markers of a second panel may each exhibit a decrease in quantity or activity in psoriatic tissue as compared to non-psoriatic tissue. Similarly, different panels of markers may be composed of markers from different tissues (*e.g.*, blood (Table 10) or skin cells (Tables 1 and 2)), or may represent different components of a psoriatic disease state (*e.g.*, a more generalized inflammation reaction (Tables 1, 2, or 4) or psoriasis itself (Table 3)). Panels of the markers of the invention are set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7. It will be apparent to one skilled in the art that the methods of the invention may be practiced with any one of the panels set forth in Figures 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, or any portion or combination thereof.

It will also be appreciated by one skilled in the art that the panels of markers of the invention may conveniently be provided on solid supports. For example, polynucleotides, such as mRNA, may be coupled to an array (*e.g.*, a GeneChip array for hybridization analysis), to a resin (*e.g.*, a resin which can be packed into a column for column chromatography), or a matrix (*e.g.*, a nitrocellulose matrix for northern blot analysis). The immobilization of molecules complementary to the marker(s), either covalently or noncovalently, permits a discrete analysis of the presence or activity of each marker in a sample. In an array, for example, polynucleotides complementary to each member of a panel of markers may individually be attached to different, known locations on the array. The array may be hybridized with, for example, polynucleotides extracted from a skin cell sample from a subject. The hybridization of polynucleotides from the sample with the array at any location on the array can be detected, and thus the presence or quantity of the marker in the sample can be ascertained. In a preferred embodiment, a "GeneChip" array is employed (Affymetrix). Similarly, Western analyses may be performed on immobilized antibodies specific for different polypeptide markers hybridized to a protein sample from a subject.

It will also be apparent to one skilled in the art that the entire marker protein or nucleic acid molecule need not be conjugated to the support; a portion of the marker of sufficient length for detection purposes (*e.g.*, for hybridization), for example, a portion

of the marker which is 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 100 or more nucleotides or amino acids in length may be sufficient for detection purposes.

The nucleic acid and protein markers of the invention may be isolated from any tissue or cell of a subject. In a preferred embodiment, the tissue is skin tissue or blood tissue. However, it will be apparent to one skilled in the art that other tissue samples, including bodily fluids (*e.g.*, urine, bile, serum, lymph, saliva, mucus and pus) and other tissue samples may also serve as sources from which the markers of the invention may be isolated, or in which the presence, activity, and/or quantity of the markers of the invention may be assessed. The tissue samples containing one or more of the markers themselves may be useful in the methods of the invention, and one skilled in the art will be cognizant of the methods by which such samples may be conveniently obtained, stored, and/or preserved.

Several markers were known prior to the invention to be associated with psoriasis. These markers are set forth in Table 9. These markers are not included with the markers of the invention. However, these markers may be conveniently be used in combination with the markers of the invention in the methods, panels, and kits of the invention.

In another aspect, the invention provides methods of making an isolated hybridoma which produces an antibody useful for assessing whether a patient is afflicted with psoriasis or a TH1-associated condition. In this method, a protein corresponding to a marker of the invention is isolated (*e.g.*, by purification from a cell in which it is expressed or by transcription and translation of a nucleic acid encoding the protein in vivo or in vitro using known methods. A vertebrate, preferably a mammal such as a mouse, rat, rabbit, or sheep, is immunized using the isolated protein or protein fragment. The vertebrate may optionally (and preferably) be immunized at least one additional time with the isolated protein or protein fragment, so that the vertebrate exhibits a robust immune response to the protein or protein fragment. Splenocytes are isolated from the immunized vertebrate and fused with an immortalized cell line to form hybridomas, using any of a variety of methods well known in the art. Hybridomas formed in this manner are then screened using standard methods to identify one or more hybridomas which produce an antibody which specifically binds with the protein or protein fragment. The invention also includes hybridomas made by this method and antibodies made using such hybridomas.

The invention provides methods of diagnosing psoriasis or a TH1-associated condition, or risk of developing psoriasis or a TH1-associated condition in a subject. These methods involve isolating a sample from a subject (*e.g.*, a sample containing skin cells or blood cells), detecting the presence, quantity, and/or activity of one or more

markers of the invention in the sample relative to a second sample from a subject known not to have psoriasis or a TH1-associated condition, or from a tissue in the same subject known not to be altered by the presence of psoriasis or a TH1-associated condition in the subject. The levels of markers in the two samples are compared, and a significant
5 increase or decrease in one or more markers in the test sample indicates the presence or risk of presence of psoriasis or a TH1-associated condition in the subject.

The invention also provides methods of assessing the severity of psoriasis or a TH1-associated condition in a subject. These methods involve isolating a sample from a subject (*e.g.*, a sample containing skin cells or blood cells), detecting the presence,
10 quantity, and/or activity of one or more markers of the invention in the sample relative to a second sample from a subject known not to have psoriasis or a TH1-associated condition, or from a tissue in the same subject known not to be affected by the presence of psoriasis or a TH1-associated condition. The levels of markers in the two samples are compared, and a significant increase or decrease in one or more markers in the test
15 sample is correlated with the degree of severity of psoriasis or a TH1-associated condition in the subject.

The invention also provides methods of treating (*e.g.*, inhibiting) psoriasis or a TH1-associated condition in a subject. These methods involve isolating a sample from a subject (*e.g.*, a sample containing skin cells or blood cells), detecting the presence,
20 quantity, and/or activity of one or more markers of the invention in the sample relative to a second sample from a subject known not to have psoriasis or a TH1-associated condition, or from a tissue in the same subject known not to be affected by the presence of psoriasis or TH1-associated condition. The levels of markers in the two samples are compared, and significant increases or decreases in one or more markers in the test
25 sample relative to the control sample are observed. For markers that are significantly decreased in expression or activity, the subject may be administered that expressed marker protein, or may be treated by the introduction of mRNA or DNA corresponding to the decreased marker (*e.g.*, by gene therapy), to thereby increase the levels of the marker protein in the subject. For markers that are significantly increased in expression
30 or activity, the subject may be administered mRNA or DNA antisense to the increased marker (*e.g.*, by gene therapy), or may be administered antibodies specific for the marker protein, to thereby decrease the levels of the marker protein in the subject. In this manner, the subject may be treated for psoriasis or a TH1-associated condition.

The invention also provides methods of preventing the development of psoriasis
35 or a TH1-associated condition in a subject. These methods involve, for markers that are significantly decreased in expression or activity, the administration of that marker protein, or the introduction of mRNA or DNA corresponding to the decreased marker

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(*e.g.*, by gene therapy), to thereby increase the levels of the marker protein in the subject. For markers that are significantly increased in expression or activity, the subject may be administered mRNA or DNA antisense to the increased marker (*e.g.*, by gene therapy), or may be administered antibodies specific for the marker protein, to thereby decrease the levels of the marker protein in the subject. In this manner, the development of psoriasis or a TH1-associated condition in a subject may be prevented.

The invention also provides methods of assessing a treatment or therapy for psoriasis or a TH1-associated condition in a subject. These methods involve isolating a sample from a subject (*e.g.*, a sample containing skin cells or blood cells) suffering from psoriasis or a TH1-associated condition who is undergoing a treatment or therapy, detecting the presence, quantity, and/or activity of one or more markers of the invention in the first sample relative to a second sample from a subject afflicted with psoriasis or a TH1-associated condition who is not undergoing any treatment or therapy for the condition, and also relative to a third sample from a subject unaffected by psoriasis or a TH1-associated condition or from a tissue in the same subject known not to be affected by the presence of psoriasis or a TH1-associated condition. The levels of markers in the three samples are compared, and significant increases or decreases in one or more markers in the first sample relative to the other samples are observed, and correlated with the presence, risk of presence, or severity of psoriasis or a TH1-associated condition. By assessing whether psoriasis or a TH1-associated condition has been lessened or alleviated in the sample, the ability of the treatment or therapy to treat psoriasis or a TH1-associated condition is also determined.

The invention also provides pharmaceutical compositions for the treatment of psoriasis or a TH1-associated condition. These compositions may include a marker protein and/or nucleic acid of the invention (*e.g.*, for those markers which are decreased in quantity or activity in psoriatic tissue versus nonpsoriatic tissue), and can be formulated as described herein. Alternately, these compositions may include an antibody which specifically binds to a marker protein of the invention and/or an antisense nucleic acid molecule which is complementary to a marker nucleic acid of the invention (*e.g.*, for those markers which are increased in quantity or activity in psoriatic tissue versus nonpsoriatic tissue), and can be formulated as described herein.

The invention also provides kits for assessing the presence of psoriatic cells or cells participating in a TH1-associated condition in a sample (*e.g.*, a sample from a subject at risk for psoriasis or a TH1-associated condition), the kit comprising an antibody, wherein the antibody specifically binds with a protein corresponding to a marker selected from the group consisting of the markers listed in Tables 1-10 and 12,

and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7.

5 The invention further provides kits for assessing the presence of psoriatic cells or cells participating in a TH1-associated condition in a sample from a subject (*e.g.*, a subject at risk for psoriasis or a TH1-associated condition), the kit comprising a nucleic acid probe wherein the probe specifically binds with a transcribed polynucleotide corresponding to a marker selected from the group consisting of the markers listed in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7.

10 The invention further provides kits for assessing the suitability of each of a plurality of compounds for inhibiting psoriasis or a TH1-associated condition in a subject. Such kits include a plurality of compounds to be tested, and a reagent for assessing expression of a marker selected from the group consisting of one or more of the markers set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7.

15 Modifications to the above-described compositions and methods of the invention, according to standard techniques, will be readily apparent to one skilled in the art and are meant to be encompassed by the invention.

20 To facilitate an understanding of the present invention, a number of terms and phrases are defined below:

As used herein, the terms "polynucleotide" and "oligonucleotide" are used interchangeably, and include polymeric forms of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown.

25 The following are non-limiting examples of polynucleotides: a gene or gene fragment, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. The term also includes both double- and single-stranded molecules. Unless otherwise
35 specified or required, any embodiment of this invention that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

5 inputted into databases in a computer having a central processing unit and used for
bioinformatics applications such as functional genomics and homology searching.

10 identify larger fragments or full-length coding sequences of the gene with which they are
associated. Methods of isolating larger fragment sequences are known to those of skill
in the art, some of which are described herein.

A “gene product” includes an amino acid (e.g., peptide or polypeptide) generated when a gene is transcribed and translated.

15 As used herein, a “polynucleotide corresponds to” another (a first) polynucleotide if it is related to the first polynucleotide by any of the following relationships:

1) The second polynucleotide comprises the first polynucleotide and the second polynucleotide encodes a gene product.

20 2) The second polynucleotide is 5' or 3' to the first polynucleotide in cDNA, RNA, genomic DNA, or fragments of any of these polynucleotides. For example, a second polynucleotide may be a fragment of a gene that includes the first and second polynucleotides. The first and second polynucleotides are related in that they are components of the gene coding for a gene product, such as a protein or antibody.

25 However, it is not necessary that the second polynucleotide comprises or overlaps with
the first polynucleotide to be encompassed within the definition of “corresponding to” as
used herein. For example, the first polynucleotide may be a fragment of a 3’
untranslated region of the second polynucleotide. The first and second polynucleotide
may be fragments of a gene coding for a gene product. The second polynucleotide may
30 be an exon of the gene while the first polynucleotide may be an intron of the gene.

3) The second polynucleotide is the complement of the first polynucleotide.

A “probe” when used in the context of polynucleotide manipulation includes an oligonucleotide that is provided as a reagent to detect a target present in a sample of interest by hybridizing with the target. Usually, a probe will comprise a label or a means by which a label can be attached, either before or subsequent to the hybridization reaction. Suitable labels include, but are not limited to radioisotopes, fluorochromes, chemiluminescent compounds, dyes, and proteins, including enzymes.

A "primer" includes a short polynucleotide, generally with a free 3'-OH group that binds to a target or "template" present in a sample of interest by hybridizing with the target, and thereafter promoting polymerization of a polynucleotide complementary to the target. A "polymerase chain reaction" ("PCR") is a reaction in which replicate
5 copies are made of a target polynucleotide using a "pair of primers" or "set of primers" consisting of "upstream" and a "downstream" primer, and a catalyst of polymerization, such as a DNA polymerase, and typically a thermally-stable polymerase enzyme. Methods for PCR are well known in the art, and are taught, for example, in MacPherson et al. , IRL Press at Oxford University Press (1991)). All processes of producing
10 replicate copies of a polynucleotide, such as PCR or gene cloning, are collectively referred to herein as "replication". A primer can also be used as a probe in hybridization reactions, such as Southern or Northern blot analyses (see, e.g., Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY,*
15 1989).

The term "cDNAs" includes complementary DNA, that is mRNA molecules present in a cell or organism made into cDNA with an enzyme such as reverse transcriptase. A "cDNA library" includes a collection of mRNA molecules present in a cell or organism, converted into cDNA molecules with the enzyme reverse transcriptase,
20 then inserted into "vectors" (other DNA molecules that can continue to replicate after addition of foreign DNA). Exemplary vectors for libraries include bacteriophage, viruses that infect bacteria (e.g., lambda phage). The library can then be probed for the specific cDNA (and thus mRNA) of interest.

A "gene delivery vehicle" includes a molecule that is capable of inserting one
25 or more polynucleotides into a host cell. Examples of gene delivery vehicles are liposomes, biocompatible polymers, including natural polymers and synthetic polymers; lipoproteins; polypeptides; polysaccharides; lipopolysaccharides; artificial viral envelopes; metal particles; and bacteria, viruses and viral vectors, such as baculovirus, adenovirus, and retrovirus, bacteriophage, cosmid, plasmid, fungal vector and other
30 recombination vehicles typically used in the art which have been described for replication and/or expression in a variety of eukaryotic and prokaryotic hosts. The gene delivery vehicles may be used for replication of the inserted polynucleotide, gene therapy as well as for simply polypeptide and protein expression.

A "vector" includes a self-replicating nucleic acid molecule that transfers an
35 inserted polynucleotide into and/or between host cells. The term is intended to include vectors that function primarily for insertion of a nucleic acid molecule into a cell, replication vectors that function primarily for the replication of nucleic acid and

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expression vectors that function for transcription and/or translation of the DNA or RNA. Also intended are vectors that provide more than one of the above function.

A "host cell" is intended to include any individual cell or cell culture which can be or has been a recipient for vectors or for the incorporation of exogenous nucleic acid molecules, polynucleotides and/or proteins. It also is intended to include progeny of a single cell. The progeny may not necessarily be completely identical (in morphology or in genomic or total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. The cells may be prokaryotic or eukaryotic, and include but are not limited to bacterial cells, yeast cells, insect cells, animal cells, and mammalian cells, *e.g.*, murine, rat, simian or human cells.

The term "genetically modified" includes a cell containing and/or expressing a foreign gene or nucleic acid sequence which in turn modifies the genotype or phenotype of the cell or its progeny. This term includes any addition, deletion, or disruption to a cell's endogenous nucleotides.

As used herein, "expression" includes the process by which polynucleotides are transcribed into mRNA and translated into peptides, polypeptides, or proteins. If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA, if an appropriate eukaryotic host is selected. Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG (Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). Similarly, a eukaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors can be obtained commercially or assembled by the sequences described in methods well known in the art, for example, the methods described below for constructing vectors in general.

"Differentially expressed", as applied to a gene, includes the differential production of mRNA transcribed from a gene or a protein product encoded by the gene. A differentially expressed gene may be overexpressed or underexpressed as compared to the expression level of a normal or control cell. In one aspect, it includes a differential that is 2.5 times, preferably 5 times or preferably 10 times higher or lower than the expression level detected in a control sample. The term "differentially expressed" also includes nucleotide sequences in a cell or tissue which are expressed where silent in a control cell or not expressed where expressed in a control cell.

The term "polypeptide" includes a compound of two or more subunit amino acids, amino acid analogs, or peptidomimetics. The subunits may be linked by peptide bonds. In another embodiment, the subunit may be linked by other bonds, *e.g.*, ester, ether, etc. As used herein the term "amino acid" includes either natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics. A peptide of three or more amino acids is commonly referred to as an oligopeptide. Peptide chains of greater than three or more amino acids are referred to as a polypeptide or a protein.

"Hybridization" includes a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogsteen binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PCR reaction, or the enzymatic cleavage of a polynucleotide by a ribozyme.

Hybridization reactions can be performed under conditions of different "stringency". The stringency of a hybridization reaction includes the difficulty with which any two nucleic acid molecules will hybridize to one another. Under stringent conditions, nucleic acid molecules at least 60%, 65%, 70%, 75% identical to each other remain hybridized to each other, whereas molecules with low percent identity cannot remain hybridized. A preferred, non-limiting example of highly stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50°C, preferably at 55°C, more preferably at 60°C, and even more preferably at 65°C.

When hybridization occurs in an antiparallel configuration between two single-stranded polynucleotides, the reaction is called "annealing" and those polynucleotides are described as "complementary". A double-stranded polynucleotide can be "complementary" or "homologous" to another polynucleotide, if hybridization can occur between one of the strands of the first polynucleotide and the second. "Complementarity" or "homology" (the degree that one polynucleotide is complementary with another) is quantifiable in terms of the proportion of bases in opposing strands that are expected to hydrogen bond with each other, according to generally accepted base-pairing rules.

An "antibody" includes an immunoglobulin molecule capable of binding an epitope present on an antigen. As used herein, the term encompasses not only intact

immunoglobulin molecules such as monoclonal and polyclonal antibodies, but also anti-idotypic antibodies, mutants, fragments, fusion proteins, bi-specific antibodies, humanized proteins, and modifications of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity.

As used herein, the term “psoriasis” includes a noncontagious skin disorder that most commonly appears as inflamed swollen skin lesions covered with silvery white scale. The term psoriasis encompasses various manifestations of psoriasis, such as plaque psoriasis, pustular psoriasis, erythrodermic psoriasis, guttate psoriasis, and inverse psoriasis. Also encompassed by this term is psoriatic arthritis.

10 As used herein, the term “TH1-associated condition” includes diseases and conditions in which, like psoriasis, T-helper 1 (TH1) cells are thought to play a role, in either the origin or progression of the disease. Examples of TH1-associated conditions include, but are not limited to, rheumatoid arthritis, multiple sclerosis, and Crohn’s disease.

As used herein, the term “psoriatic tissue” or “psoriatic cell” or “involved tissue” or “involved cell” includes a tissue from a subject afflicted with psoriasis, where the tissue itself is involved in the symptomology of psoriasis. An example of a psoriatic tissue is skin tissue, *e.g.*, epithelial tissue, taken from a psoriatic lesion, scale, or pustule. A “non-psoriatic tissue” or “non-psoriatic cell” includes a tissue or cell which either is from a subject not afflicted with psoriasis, or which is from a subject afflicted with psoriasis, but in which the tissue or cell is taken from an area of the subject that is not displaying the symptoms of psoriasis. For example, a non-psoriatic tissue would include skin cells from an afflicted subject, where the cells are taken from a section of the skin which is not affected by psoriatic lesions, scaling, or pustules, and would also include epithelial cells taken from an unafflicted subject.

As used herein, the term “marker” includes a polynucleotide or polypeptide molecule which is present or absent, or increased or decreased in quantity or activity in subjects afflicted with psoriasis or a TH1-associated condition, or in cells involved in psoriasis or a TH1-associated condition. The relative change in quantity or activity of the marker is correlated with the incidence or risk of incidence of psoriasis or a TH1-associated condition.

As used herein, the term “panel of markers” includes a group of markers, the quantity or activity of each member of which is correlated with the incidence or risk of incidence of psoriasis or a TH1-associated condition. In certain embodiments, a panel of markers may include only those markers which are either increased or decreased in quantity or activity in subjects afflicted with or cells involved in psoriasis or a TH1-associated condition. In other embodiments, a panel of markers may include only those

markers present in a specific tissue type which are correlated with the incidence or risk of incidence of psoriasis or a TH1-associated condition.

5 Various aspects of the invention are described in further detail in the following subsections:

I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that either themselves are the genetic markers (*e.g.*, mRNA) of the invention, or which encode the polypeptide markers of the invention, or fragments thereof. Another aspect of the invention pertains to isolated nucleic acid fragments sufficient for use as hybridization probes to identify the nucleic acid molecules encoding the markers of the invention in a sample, as well as nucleotide fragments for use as PCR primers for the amplification or mutation of the nucleic acid molecules which encode the markers of the invention. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated marker nucleic acid molecule of the invention, or nucleic acid molecule encoding a polypeptide marker of the invention, can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

35 A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of one of the genes set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, or

a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequence of one of the genes set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7 as a hybridization probe, a marker
5 gene of the invention or a nucleic acid molecule encoding a polypeptide marker of the invention can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

10 A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to marker nucleotide sequences, or
15 nucleotide sequences encoding a marker of the invention can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence of a marker of the invention (*e.g.*, a gene set forth in Tables 1-10 and 12, and
20 Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7), or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to such a nucleotide sequence is one which is sufficiently complementary to the nucleotide sequence such that it can hybridize to the nucleotide sequence, thereby forming a stable duplex.

25 The nucleic acid molecule of the invention, moreover, can comprise only a portion of the nucleic acid sequence of a marker nucleic acid of the invention, or a gene encoding a marker polypeptide of the invention, for example, a fragment which can be used as a probe or primer. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide
30 sequence that hybridizes under stringent conditions to at least about 7 or 15, preferably about 20 or 25, more preferably about 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 400 or more consecutive nucleotides of a marker nucleic acid, or a nucleic acid encoding a marker polypeptide of the invention.

Probes based on the nucleotide sequence of a marker gene or of a nucleic acid
35 molecule encoding a marker polypeptide of the invention can be used to detect transcripts or genomic sequences corresponding to the marker gene(s) and/or marker polypeptide(s) of the invention. In preferred embodiments, the probe comprises a label

group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress (*e.g.*, over- or under-express) a marker polypeptide of the invention, or which have greater or fewer copies of a marker gene of the invention. For example, a level of a marker polypeptide-encoding nucleic acid in a sample of cells from a subject may be detected, the amount of mRNA transcript of a gene encoding a marker polypeptide may be determined, or the presence of mutations or deletions of a marker gene of the invention may be assessed.

The invention further encompasses nucleic acid molecules that differ from the nucleic acid sequences of the genes set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, due to degeneracy of the genetic code and which thus encode the same proteins as those encoded by the genes shown in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7.

In addition to the nucleotide sequences of the genes set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the proteins encoded by the genes set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7 may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the genes set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7 may exist among individuals within a population due to natural allelic variation. An allele is one of a group of genes which occur alternatively at a given genetic locus. In addition it will be appreciated that DNA polymorphisms that affect RNA expression levels can also exist that may affect the overall expression level of that gene (*e.g.*, by affecting regulation or degradation). As used herein, the phrase "allelic variant" includes a nucleotide sequence which occurs at a given locus or to a polypeptide encoded by the nucleotide sequence. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a marker polypeptide of the invention.

Nucleic acid molecules corresponding to natural allelic variants and homologues of the marker genes, or genes encoding the marker proteins of the invention can be isolated based on their homology to the genes set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

Nucleic acid molecules corresponding to natural allelic variants and homologues of the marker genes of the invention can further be isolated by mapping to the same chromosome or locus as the marker genes or genes encoding the marker proteins of the invention.

5 In another embodiment, an isolated nucleic acid molecule of the invention is at least 15, 20, 25, 30, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000 or more nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule corresponding to a nucleotide sequence of a marker gene or gene
10 encoding a marker protein of the invention. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about
15 85% or 90% homologous to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more
20 washes in 0.2 X SSC, 0.1% SDS at 50°C, preferably at 55°C, more preferably at 60°C, and even more preferably at 65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of one of the genes set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7 corresponds to a naturally-occurring nucleic acid
25 molecule. As used herein, a "naturally-occurring" nucleic acid molecule includes an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In addition to naturally-occurring allelic variants of the marker gene and gene encoding a marker protein of the invention sequences that may exist in the population,
30 the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of the marker genes or genes encoding the marker proteins of the invention, thereby leading to changes in the amino acid sequence of the encoded proteins, without altering the functional activity of these proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid
35 residues can be made. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of a protein without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For

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example, amino acid residues that are conserved among allelic variants or homologs of a gene (*e.g.*, among homologs of a gene from different species) are predicted to be particularly unamenable to alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules
5 encoding a marker protein of the invention that contain changes in amino acid residues that are not essential for activity. Such proteins differ in amino acid sequence from the marker proteins encoded by the genes set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, yet retain biological activity. In one embodiment, the protein comprises an amino acid sequence at
10 least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to a marker protein of the invention.

An isolated nucleic acid molecule encoding a protein homologous to a marker protein of the invention can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of the gene encoding the
15 marker protein, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into the genes of the invention (*e.g.*, a gene set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7) by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative
20 amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine),
25 acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be
30 introduced randomly along all or part of a coding sequence of a gene of the invention, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

35 Another aspect of the invention pertains to isolated nucleic acid molecules which are antisense to the marker genes and genes encoding marker proteins of the invention. An "antisense" nucleic acid comprises a nucleotide sequence which is

complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire coding strand of a gene of the invention (*e.g.*, a gene set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7), or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence of the invention. The term "coding region" includes the region of the nucleotide sequence comprising codons which are translated into amino acid. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence of the invention. The term "noncoding region" includes 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of an mRNA corresponding to a gene of the invention, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil,



transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a marker protein of the invention to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site (*e.g.*, in skin). Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

25 In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-

30 methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave mRNA transcripts of the genes of the invention (*e.g.*, a gene set forth

in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7) to thereby inhibit translation of this mRNA. A ribozyme having specificity for a marker protein-encoding nucleic acid can be designed based upon the nucleotide sequence of a gene of the invention, disclosed herein. For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a marker protein-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively, mRNA transcribed from a gene of the invention can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

Alternatively, expression of a gene of the invention (*e.g.*, a gene set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7) can be inhibited by targeting nucleotide sequences complementary to the regulatory region of these genes (*e.g.*, the promoter and/or enhancers) to form triple helical structures that prevent transcription of the gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

In yet another embodiment, the nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* *Proc. Natl. Acad. Sci.* 93: 14670-675.

PNAs can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of the nucleic acid molecules of the invention (*e.g.*, a gene set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group

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III, 6 Group IV, and 7) can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (e.g., S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *supra*).

In another embodiment, PNAs can be modified, (e.g., to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of the nucleic acid molecules of the invention can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (e.g., RNase H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, e.g., 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, e.g., Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, e.g., Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent). Finally, the oligonucleotide may be detectably labeled, either such that the label is detected by the addition of another reagent (e.g., a substrate for an enzymatic label), or is

detectable immediately upon hybridization of the nucleotide (*e.g.*, a radioactive label or a fluorescent label (*e.g.*, a molecular beacon, as described in U.S. Patent 5,876,930.

II. Isolated Proteins and Antibodies

5 One aspect of the invention pertains to isolated marker proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-marker protein antibodies. In one embodiment, native marker proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, marker
10 proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a marker protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

 An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or
15 tissue source from which the marker protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of marker protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of
20 cellular material" includes preparations of marker protein having less than about 30% (by dry weight) of non-marker protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-marker protein, still more preferably less than about 10% of non-marker protein, and most preferably less than about 5% non-marker protein. When the marker protein or biologically active portion
25 thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

 The language "substantially free of chemical precursors or other chemicals"
30 includes preparations of marker protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of protein having less than about 30% (by dry weight) of chemical precursors or non-protein chemicals, more preferably less than about 20% chemical
35 precursors or non-protein chemicals, still more preferably less than about 10% chemical precursors or non-protein chemicals, and most preferably less than about 5% chemical precursors or non-protein chemicals.

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As used herein, a "biologically active portion" of a marker protein includes a fragment of a marker protein comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the marker protein, which include fewer amino acids than the full length marker proteins, and exhibit at least one activity of a marker protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the marker protein. A biologically active portion of a marker protein can be a polypeptide which is, for example, 10, 25, 50, 100, 200 or more amino acids in length. Biologically active portions of a marker protein can be used as targets for developing agents which modulate a marker protein-mediated activity.

In a preferred embodiment, marker protein is encoded by a gene set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7. In other embodiments, the marker protein is substantially homologous to a marker protein encoded by a gene set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, and retains the functional activity of the marker protein, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above. Accordingly, in another embodiment, the marker protein is a protein which comprises an amino acid sequence at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to the amino acid sequence encoded by a gene set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has
5 been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at
10 <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue
15 table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J.*
20 *Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to marker protein molecules of the invention. To obtain gapped
25 alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

The invention also provides chimeric or fusion marker proteins. As used herein,
30 a marker "chimeric protein" or "fusion protein" comprises a marker polypeptide operatively linked to a non-marker polypeptide. An "marker polypeptide" includes a polypeptide having an amino acid sequence encoded by a gene set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, whereas a "non-marker polypeptide" includes a polypeptide having an amino
35 acid sequence corresponding to a protein which is not substantially homologous to the marker protein, *e.g.*, a protein which is different from marker protein and which is derived from the same or a different organism. Within a marker fusion protein the

polypeptide can correspond to all or a portion of a marker protein. In a preferred embodiment, a marker fusion protein comprises at least one biologically active portion of a marker protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the marker polypeptide and the non-marker polypeptide are fused in-frame to each other. The non-marker polypeptide can be fused to the N-terminus or C-terminus of the marker polypeptide.

For example, in one embodiment, the fusion protein is a GST-marker fusion protein in which the marker sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant marker proteins.

In another embodiment, the fusion protein is a marker protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of marker proteins can be increased through use of a heterologous signal sequence. Such signal sequences are well known in the art.

The marker fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*, as described herein. The marker fusion proteins can be used to affect the bioavailability of a marker protein substrate. Use of marker fusion proteins may be useful therapeutically for the treatment of disorders (e.g., psoriasis or a TH1-associated condition) caused by, for example, (i) aberrant modification or mutation of a gene encoding a marker protein; (ii) mis-regulation of the marker protein-encoding gene; and (iii) aberrant post-translational modification of a marker protein.

Moreover, the marker-fusion proteins of the invention can be used as immunogens to produce anti-marker protein antibodies in a subject, to purify marker protein ligands and in screening assays to identify molecules which inhibit the interaction of a marker protein with a marker protein substrate.

Preferably, a marker chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric

gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A marker protein-encoding nucleic acid can be cloned into such an expression vector such that the
5 fusion moiety is linked in-frame to the marker protein.

A signal sequence can be used to facilitate secretion and isolation of the secreted protein or other proteins of interest. Signal sequences are typically characterized by a core of hydrophobic amino acids which are generally cleaved from the mature protein during secretion in one or more cleavage events. Such signal peptides contain
10 processing sites that allow cleavage of the signal sequence from the mature proteins as they pass through the secretory pathway. Thus, the invention pertains to the described polypeptides having a signal sequence, as well as to polypeptides from which the signal sequence has been proteolytically cleaved (*i.e.*, the cleavage products). In one embodiment, a nucleic acid sequence encoding a signal sequence can be operably linked
15 in an expression vector to a protein of interest, such as a protein which is ordinarily not secreted or is otherwise difficult to isolate. The signal sequence directs secretion of the protein, such as from a eukaryotic host into which the expression vector is transformed, and the signal sequence is subsequently or concurrently cleaved. The protein can then be readily purified from the extracellular medium by art recognized methods.
20 Alternatively, the signal sequence can be linked to the protein of interest using a sequence which facilitates purification, such as with a GST domain.

The present invention also pertains to variants of the marker proteins of the invention which function as either agonists (mimetics) or as antagonists to the marker proteins. Variants of the marker proteins can be generated by mutagenesis, *e.g.*, discrete
25 point mutation or truncation of a marker protein. An agonist of the marker proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a marker protein. An antagonist of a marker protein can inhibit one or more of the activities of the naturally occurring form of the marker protein by, for example, competitively modulating an activity of a marker protein. Thus, specific
30 biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the marker protein.

Variants of a marker protein which function as either marker protein agonists
35 (mimetics) or as marker protein antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of a marker protein for marker protein agonist or antagonist activity. In one embodiment, a variegated library of marker protein

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variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of marker protein variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential marker protein sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of marker protein sequences therein. There are a variety of methods which can be used to produce libraries of potential marker protein variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential marker protein sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477).

In addition, libraries of fragments of a protein coding sequence corresponding to a marker protein of the invention can be used to generate a variegated population of marker protein fragments for screening and subsequent selection of variants of a marker protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a marker protein coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the marker protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of

functional mutants in the libraries, can be used in combination with the screening assays to identify marker variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

5 An isolated marker protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind marker proteins using standard techniques for polyclonal and monoclonal antibody preparation. A full-length marker protein can be used or, alternatively, the invention provides antigenic peptide fragments of these proteins for use as immunogens. The antigenic peptide of a marker protein comprises at least 8 amino acid residues of an amino acid sequence encoded by a gene set forth in
10 Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7, and encompasses an epitope of a marker protein such that an antibody raised against the peptide forms a specific immune complex with the marker protein. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino
15 acid residues, and most preferably at least 30 amino acid residues.

Preferred epitopes encompassed by the antigenic peptide are regions of the marker protein that are located on the surface of the protein, *e.g.*, hydrophilic regions, as well as regions with high antigenicity.

A marker protein immunogen typically is used to prepare antibodies by
20 immunizing a suitable subject, (*e.g.*, rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed marker protein or a chemically synthesized marker polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a
25 suitable subject with an immunogenic marker protein preparation induces a polyclonal anti-marker protein antibody response.

Accordingly, another aspect of the invention pertains to anti-marker protein antibodies. The term "antibody" as used herein includes immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that
30 contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as a marker protein. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind to marker proteins. The term
35 "monoclonal antibody" or "monoclonal antibody composition", as used herein, includes a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope. A monoclonal antibody

composition thus typically displays a single binding affinity for a particular marker protein with which it immunoreacts.

Polyclonal anti-marker protein antibodies can be prepared as described above by immunizing a suitable subject with a marker protein of the invention. The anti-
5 marker protein antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized marker protein. If desired, the antibody molecules directed against marker proteins can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as protein A chromatography, to obtain the IgG
10 fraction. At an appropriate time after immunization, e.g., when the anti-marker protein antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.* (1976) *Proc. Natl. Acad. Sci. USA* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing
20 monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.*, 54:387-402; M. L. Gefter *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes)
25 from a mammal immunized with a marker protein immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds to a marker protein of the invention.

Any of the many well known protocols used for fusing lymphocytes and
30 immortalized cell lines can be applied for the purpose of generating an anti-marker protein monoclonal antibody (see, e.g., G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al.* *Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful.
35 Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of

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the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind to a marker protein, *e.g.*, using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-marker protein antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with marker protein to thereby isolate immunoglobulin library members that bind to a marker protein. Kits for generating and screening phage display libraries are commercially available (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619; Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.* PCT International Publication WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling *et al.* PCT International Publication WO 93/01288; McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT International Publication No. WO 92/09690; Ladner *et al.* PCT International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al.* *Nature* (1990) 348:552-554.

Additionally, recombinant anti-marker protein antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of

the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496;

- 5 Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA*
10 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

- 15 Completely human antibodies are particularly desirable for therapeutic treatment of human subjects. Such antibodies can be produced using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chain genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen, *e.g.*, all or a portion of a
20 polypeptide corresponding to a marker of the invention. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA
25 and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995) *Int. Rev. Immunol.* 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, *e.g.*, U.S. Patent 5,625,126; U.S. Patent 5,633,425; U.S. Patent 5,569,825; U.S. Patent 5,661,016; and U.S. Patent
30 5,545,806. In addition, companies such as Abgenix, Inc. (Freemont, CA), can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

- Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected
35 non-human monoclonal antibody, *e.g.*, a murine antibody, is used to guide the selection of a completely human antibody recognizing the same epitope (Jespers *et al.*, 1994, *Bio/technology* 12:899-903).

An anti-marker protein antibody (*e.g.*, monoclonal antibody) can be used to isolate a marker protein of the invention by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-marker protein antibody can facilitate the purification of natural marker proteins from cells and of recombinantly produced marker proteins expressed in host cells. Moreover, an anti-marker protein antibody can be used to detect marker protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the marker protein. Anti-marker protein antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a marker protein of the invention (or a portion thereof). As used herein, the term "vector" includes a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which includes a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant

DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., marker proteins, mutant forms of marker proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of marker proteins in prokaryotic or eukaryotic cells. For example, marker proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion
5 vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from
10 the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST),
15 maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in marker activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for marker proteins, for example.

Examples of suitable inducible non-fusion *E. coli* expression vectors include
20 pTrc (Amann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion
25 promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to
30 express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those
35 preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the marker protein expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, *et al.*, (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (Invitrogen Corp, San Diego, CA).

Alternatively, marker proteins of the invention can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, Sf9 cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.*

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to mRNA corresponding to a gene of the invention (e.g., a gene set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7). Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a nucleic acid molecule of the invention is introduced, e.g., a gene set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7 within a recombinant expression vector or a nucleic acid molecule of the invention containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a marker protein of the invention can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated

transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (*Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

5 For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred
10 selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding a marker protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable
15 marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) a marker protein. Accordingly, the invention further provides methods for producing a marker protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of
20 invention (into which a recombinant expression vector encoding a marker protein has been introduced) in a suitable medium such that a marker protein of the invention is produced. In another embodiment, the method further comprises isolating a marker protein from the medium or the host cell.

The host cells of the invention can also be used to produce non-human transgenic
25 animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which marker-protein-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous sequences encoding a marker protein of the invention have been introduced into their genome or homologous recombinant animals in which
30 endogenous sequences encoding the marker proteins of the invention have been altered. Such animals are useful for studying the function and/or activity of a marker protein and for identifying and/or evaluating modulators of marker protein activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a
35 transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops

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and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous gene of the invention (e.g., a gene set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7) has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

10 A transgenic animal of the invention can be created by introducing a marker-encoding nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the
15 transgene. A tissue-specific regulatory sequence(s) can be operably linked to a transgene to direct expression of a marker protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent
20 No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of a transgene of the invention in its genome and/or expression of mRNA corresponding to a gene of the invention in tissues
25 or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a marker protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which
30 contains at least a portion of a gene of the invention into which a deletion, addition or substitution has been introduced to thereby alter, e.g., functionally disrupt, the gene. The gene can be a human gene, but more preferably, is a non-human homologue of a human gene of the invention (e.g., a gene set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7). For
35 example, a mouse gene can be used to construct a homologous recombination nucleic acid molecule, e.g., a vector, suitable for altering an endogenous gene of the invention in the mouse genome. In a preferred embodiment, the homologous recombination nucleic

acid molecule is designed such that, upon homologous recombination, the endogenous gene of the invention is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the homologous recombination nucleic acid molecule can be designed such that, upon homologous recombination, the endogenous gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous marker protein). In the homologous recombination nucleic acid molecule, the altered portion of the gene of the invention is flanked at its 5' and 3' ends by additional nucleic acid sequence of the gene of the invention to allow for homologous recombination to occur between the exogenous gene carried by the homologous recombination nucleic acid molecule and an endogenous gene in a cell, *e.g.*, an embryonic stem cell. The additional flanking nucleic acid sequence is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the homologous recombination nucleic acid molecule (see, *e.g.*, Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The homologous recombination nucleic acid molecule is introduced into a cell, *e.g.*, an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced gene has homologously recombined with the endogenous gene are selected (see *e.g.*, Li, E. *et al.* (1992) *Cell* 69:915). The selected cells can then be injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras (see *e.g.*, Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination nucleic acid molecules, *e.g.*, vectors, or homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos.: WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.*

In another embodiment, transgenic non-human animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, *e.g.*, Lakso *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the

FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of

5 "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* (1997) *Nature* 385:810-

10 813 and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such

15 that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

IV. Pharmaceutical Compositions

20 The nucleic acid molecules of the invention (*e.g.*, the genes set forth in Tables 1-10 and 12, and Figures 3, 4A, 4B, 5B, 5C, 5D, 6 Group I, 6 Group II, 6 Group III, 6 Group IV, and 7), fragments of marker proteins, and anti-marker protein antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically

25 comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically

30 active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

The invention includes methods for preparing pharmaceutical compositions for

35 modulating the expression or activity of a polypeptide or nucleic acid corresponding to a marker of the invention. Such methods comprise formulating a pharmaceutically acceptable carrier with an agent which modulates expression or activity of a polypeptide

or nucleic acid corresponding to a marker of the invention. Such compositions can further include additional active agents. Thus, the invention further includes methods for preparing a pharmaceutical composition by formulating a pharmaceutically acceptable carrier with an agent which modulates expression or activity of a polypeptide or nucleic acid corresponding to a marker of the invention and one or more additional active compounds.

The invention also provides methods (also referred to herein as "screening assays") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, peptoids, small molecules or other drugs) which (a) bind to the marker, or (b) have a modulatory (*e.g.*, stimulatory or inhibitory) effect on the activity of the marker or, more specifically, (c) have a modulatory effect on the interactions of the marker with one or more of its natural substrates (*e.g.*, peptide, protein, hormone, co-factor, or nucleic acid), or (d) have a modulatory effect on the expression of the marker. Such assays typically comprise a reaction between the marker and one or more assay components. The other components may be either the test compound itself, or a combination of test compound and a natural binding partner of the marker.

The test compounds of the present invention may be obtained from any available source, including systematic libraries of natural and/or synthetic compounds. Test compounds may also be obtained by any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; peptoid libraries (libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone which are resistant to enzymatic degradation but which nevertheless remain bioactive; see, *e.g.*, Zuckermann *et al.*, 1994, *J. Med. Chem.* 37:2678-85); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library and peptoid library approaches are limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, 1997, *Anticancer Drug Des.* 12:145).

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents;

antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a fragment of a marker protein or an anti-marker protein antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

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Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically

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acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein includes physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene

therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

V. Computer Readable Means and Arrays

Computer readable media comprising a marker(s) of the present invention is also provided. As used herein, "computer readable media" includes a medium that can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. The skilled artisan will readily appreciate how any of the presently known computer readable mediums can be used to create a manufacture comprising computer readable medium having recorded thereon a marker of the present invention.

As used herein, "recorded" includes a process for storing information on computer readable medium. Those skilled in the art can readily adopt any of the presently known methods for recording information on computer readable medium to generate manufactures comprising the markers of the present invention.

A variety of data processor programs and formats can be used to store the marker information of the present invention on computer readable medium. For example, the nucleic acid sequence corresponding to the markers can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. Any number of dataprocessor structuring formats (*e.g.*, text file or database) may be adapted in order to obtain computer readable medium having recorded thereon the markers of the present invention.

By providing the markers of the invention in computer readable form, one can routinely access the marker sequence information for a variety of purposes. For example, one skilled in the art can use the nucleotide or amino acid sequences of the invention in computer readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search

means are used to identify fragments or regions of the sequences of the invention which match a particular target sequence or target motif.

The invention also includes an array comprising a marker(s) of the present invention. The array can be used to assay expression of one or more genes in the array.

5 In one embodiment, the array can be used to assay gene expression in a tissue to ascertain tissue specificity of genes in the array. In this manner, up to about 8600 genes can be simultaneously assayed for expression. This allows a profile to be developed showing a battery of genes specifically expressed in one or more tissues.

In addition to such qualitative determination, the invention allows the quantitation of gene expression. Thus, not only tissue specificity, but also the level of expression of a battery of genes in the tissue is ascertainable. Thus, genes can be grouped on the basis of their tissue expression *per se* and level of expression in that tissue. This is useful, for example, in ascertaining the relationship of gene expression between or among tissues. Thus, one tissue can be perturbed and the effect on gene expression in a second tissue can be determined. In this context, the effect of one cell type on another cell type in response to a biological stimulus can be determined. Such a determination is useful, for example, to know the effect of cell-cell interaction at the level of gene expression. If an agent is administered therapeutically to treat one cell type but has an undesirable effect on another cell type, the invention provides an assay to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a counteracting agent or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be ascertained and counteracted.

25 In another embodiment, the array can be used to monitor the time course of
expression of one or more genes in the array. This can occur in various biological
contexts, as disclosed herein, for example development and differentiation, disease
progression, *in vitro* processes, such a cellular transformation and senescence,
autonomic neural and neurological processes, such as, for example, pain and appetite,
30 and cognitive functions, such as learning or memory.

The array is also useful for ascertaining the effect of the expression of a gene on the expression of other genes in the same cell or in different cells. This provides, for example, for a selection of alternate molecular targets for therapeutic intervention if the ultimate or downstream target cannot be regulated.

35 The array is also useful for ascertaining differential expression patterns of one or more genes in normal and diseased cells. This provides a battery of genes that could serve as a molecular target for diagnosis or therapeutic intervention.

VI. Predictive Medicine

The present invention pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenetics and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically.

- 5 Accordingly, one aspect of the present invention relates to diagnostic assays for determining marker protein and/or nucleic acid expression as well as marker protein activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with increased or decreased marker protein expression
10 or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with marker protein, nucleic acid expression or activity. For example, the number of copies of a marker gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purposes to thereby prophylactically treat an individual prior to
15 the onset of a disorder (*e.g.*, psoriasis or a TH1-associated condition) characterized by or associated with marker protein, nucleic acid expression or activity.

Another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of marker in clinical trials.

These and other agents are described in further detail in the following sections.

20

1. Diagnostic Assays

- An exemplary method for detecting the presence or absence of marker protein or nucleic acid of the invention in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an
25 agent capable of detecting the protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes the marker protein such that the presence of the marker protein or nucleic acid is detected in the biological sample. A preferred agent for detecting mRNA or genomic DNA corresponding to a marker gene or protein of the invention is a labeled nucleic acid probe capable of hybridizing to a mRNA or genomic DNA of the invention. Suitable
30 probes for use in the diagnostic assays of the invention are described herein.

- A preferred agent for detecting marker protein is an antibody capable of binding to marker protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or
35 antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is

directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect marker mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of marker mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of marker protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of marker genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of marker protein include introducing into a subject a labeled anti-marker antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample (e.g., nonpsoriatic tissue) from a control subject, contacting the control sample with a compound or agent capable of detecting marker protein, mRNA, or genomic DNA, such that the presence of marker protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of marker protein, mRNA or genomic DNA in the control sample with the presence of marker protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of marker in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting marker protein or mRNA in a biological sample; means for determining the amount of marker in the sample; and means for comparing the amount of marker in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect marker protein or nucleic acid.

2. Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant

marker expression or activity. As used herein, the term "aberrant" includes a marker expression or activity which deviates from the wild type marker expression or activity. Aberrant expression or activity includes increased or decreased expression or activity, as well as expression or activity which does not follow the wild type developmental pattern of expression or the subcellular pattern of expression. For example, aberrant marker expression or activity is intended to include the cases in which a mutation in the marker gene causes the marker gene to be under-expressed or over-expressed and situations in which such mutations result in a non-functional marker protein or a protein which does not function in a wild-type fashion, *e.g.*, a protein which does not interact with a marker ligand or one which interacts with a non-marker protein ligand.

The assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with a misregulation in marker protein activity or nucleic acid expression, such as psoriasis or a TH1-associated condition. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disorder associated with a misregulation in marker protein activity or nucleic acid expression, such as psoriasis or a TH1-associated condition. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant marker expression or activity in which a test sample is obtained from a subject and marker protein or nucleic acid (*e.g.*, mRNA or genomic DNA) is detected, wherein the presence of marker protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant marker expression or activity. As used herein, a "test sample" includes a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, blood), cell sample, or tissue (*e.g.*, skin).

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with increased or decreased marker expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder such as psoriasis or a TH1-associated condition. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with increased or decreased marker expression or activity in which a test sample is obtained and marker protein or nucleic acid expression or activity is detected (*e.g.*, wherein the abundance of marker protein or nucleic acid expression or activity is diagnostic for a subject that can

be administered the agent to treat a disorder associated with increased or decreased marker expression or activity).

The methods of the invention can also be used to detect genetic alterations in a marker gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in marker protein activity or nucleic acid expression, such as psoriasis or a TH1-associated condition. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a marker-protein, or the mis-expression of the marker gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a marker gene; 2) an addition of one or more nucleotides to a marker gene; 3) a substitution of one or more nucleotides of a marker gene, 4) a chromosomal rearrangement of a marker gene; 5) an alteration in the level of a messenger RNA transcript of a marker gene, 6) aberrant modification of a marker gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a marker gene, 8) a non-wild type level of a marker-protein, 9) allelic loss of a marker gene, and 10) inappropriate post-translational modification of a marker-protein. As described herein, there are a large number of assays known in the art which can be used for detecting alterations in a marker gene. A preferred biological sample is a tissue (e.g., skin) or blood sample isolated by conventional means from a subject.

In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g., U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, e.g., Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the marker-gene (see Abravaya *et al.* (1995) *Nucleic Acids Res.* 23:675-682). This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a marker gene under conditions such that hybridization and amplification of the marker-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or any
5 other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a marker gene from a sample cell
10 can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence
15 specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in a marker gene or a gene encoding a marker protein of the invention can be identified by hybridizing a sample and control
20 nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin, M.T. *et al.* (1996) *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in marker can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra*. Briefly, a first
25 hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays
30 complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the marker gene and detect mutations by
35 comparing the sequence of the sample marker with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxam and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560)

or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publication No. WO 94/16101; Cohen *et al.* 5 (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the marker gene or gene encoding a marker protein of the invention include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes 10 (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type marker sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to 15 basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of 20 the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

25 In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in marker cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase 30 from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a marker sequence, *e.g.*, a wild-type marker sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the 35 like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in marker genes or genes encoding a marker protein of the invention.

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For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control marker nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl. Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable

to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur
5 only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent
10 described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose subjects exhibiting symptoms or family history of a disease or illness involving a marker gene.

Furthermore, any cell type or tissue in which marker is expressed may be utilized in the prognostic assays described herein.

3. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs) on the expression or activity of a marker protein (*e.g.*, the modulation of psoriasis or a TH1-associated condition) can be applied not only in basic drug screening, but also in clinical trials. For example, the
20 effectiveness of an agent determined by a screening assay as described herein to increase marker gene expression, protein levels, or upregulate marker activity, can be monitored in clinical trials of subjects exhibiting decreased marker gene expression, protein levels, or downregulated marker activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease marker gene expression, protein levels, or
25 downregulate marker activity, can be monitored in clinical trials of subjects exhibiting increased marker gene expression, protein levels, or upregulated marker activity. In such clinical trials, the expression or activity of a marker gene, and preferably, other genes that have been implicated in, for example, a marker-associated disorder (*e.g.*, psoriasis or a TH1-associated condition) can be used as a "read out" or markers of the
30 phenotype of a particular cell.

For example, and not by way of limitation, genes, including marker genes and genes encoding a marker protein of the invention, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) which modulates marker activity (*e.g.*, identified in a screening assay as described herein) can be
35 identified. Thus, to study the effect of agents on marker-associated disorders (*e.g.*, psoriasis or a TH1-associated condition), for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of marker and other

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genes implicated in the marker-associated disorder, respectively. The levels of gene expression (*e.g.*, a gene expression pattern) can be quantified by northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of marker or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a marker protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the marker protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the marker protein, mRNA, or genomic DNA in the pre-administration sample with the marker protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of marker to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of marker to lower levels than detected, *i.e.* to decrease the effectiveness of the agent. According to such an embodiment, marker expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

C. Methods of Treatment:

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk for (or susceptible to) a disorder or having a disorder associated with aberrant marker expression or activity. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, includes the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of

how a subject's genes determine his or her response to a drug (*e.g.*, a subject's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the marker molecules of the present invention or marker modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to subjects who will most benefit from the treatment and to avoid treatment of subjects who will experience toxic drug-related side effects.

10 1. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a disease or condition (*e.g.*, psoriasis or a TH1-associated condition) associated with increased or decreased marker expression or activity, by administering to the subject a marker protein or an agent which modulates marker protein expression or at least one marker protein activity. Subjects at risk for a disease which is caused or contributed to by increased or decreased marker expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the differential marker protein expression, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of marker aberrancy (*e.g.*, increase or decrease in expression level), for example, a marker protein, marker protein agonist or marker protein antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

25 2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating marker protein expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a marker protein or agent that modulates one or more of the activities of a marker protein activity associated with the cell. An agent that modulates marker protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a marker protein (*e.g.*, a marker protein substrate), a marker protein antibody, a marker protein agonist or antagonist, a peptidomimetic of a marker protein agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more marker protein activities. Examples of such stimulatory agents include active marker protein and a nucleic acid molecule encoding marker protein that has been introduced into the cell. In another embodiment, the agent inhibits one or more marker

protein activities. Examples of such inhibitory agents include antisense marker protein nucleic acid molecules, anti-marker protein antibodies, and marker protein inhibitors. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a marker protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) marker protein expression or activity. In another embodiment, the method involves administering a marker protein or nucleic acid molecule as therapy to compensate for reduced or aberrant marker protein expression or activity.

Stimulation of marker protein activity is desirable in situations in which marker protein is abnormally downregulated and/or in which increased marker protein activity is likely to have a beneficial effect. For example, stimulation of marker protein activity is desirable in situations in which a marker is downregulated and/or in which increased marker protein activity is likely to have a beneficial effect. Likewise, inhibition of marker protein activity is desirable in situations in which marker protein is abnormally upregulated and/or in which decreased marker protein activity is likely to have a beneficial effect.

3. Pharmacogenomics

The marker protein and nucleic acid molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on marker protein activity (e.g., marker gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) marker-associated disorders (e.g., psoriasis or a TH1-associated condition) associated with aberrant marker protein activity. In conjunction with such treatment, pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a marker molecule or marker modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a marker molecule or marker modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-11) :983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43(2):254-266. In
5 general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example,
10 glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug
15 response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (*e.g.*, a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically
20 significant number of subjects taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of
25 DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of
30 genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drugs target is known (*e.g.*, a marker protein of the present invention), all
35 common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

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As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some subjects do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (*e.g.*, a marker molecule or marker modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a marker molecule or marker modulator, such as a modulator identified by one of the exemplary screening assays described herein.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and Tables are incorporated herein by reference.

EXAMPLES

**EXAMPLE 1: IDENTIFICATION AND CHARACTERIZATION OF
MARKER cDNA**

5

A. RNA Preparation for Hybridization

Labeled RNA was prepared from clones containing a T7 RNA polymerase promoter site by incorporating labeled ribonucleotides in an in vitro transcription (IVT) reaction. Either biotin-labeled or fluorescein-labeled UTP and CTP (1:3 labeled to
10 unlabeled) plus unlabeled ATP and GTP were used for the reaction with 2500 U of T7 RNA polymerase. Following the reaction, unincorporated nucleotide triphosphates were removed using a size-selective membrane (Microcon-100, Amicon, Beverly, MA). The total molar concentration of RNA was based on a measurement of the absorbance at 260 nm. Following quantitation of RNA amounts, RNA was fragmented randomly to an
15 average length of approximately 50 bases by heating at 94 °C in 40 mM Tris-acetate pH 8.1, 100 mM potassium acetate, 30 mM magnesium acetate, for 30 to 40 min. Fragmentation reduces possible interference from RNA secondary structure, and minimizes the effects of multiple interactions with closely spaced probe molecules. For material made directly from cellular RNA, cytoplasmic RNA was extracted from cells
20 by the method of Favaloro *et al.* ((1980) *Methods Enzymol.* 65: 718-749), and poly (A) RNA was isolated with an oligo dT selection step (PolyAtract, Promega, Madison, WI). RNA was amplified using a modification of the procedure described by Eberwine *et al.* ((1992) *Proc. Natl. Acad. Sci. USA* 89: 3010-3014). One microgram of poly (A) RNA was converted into double-stranded cDNA using a cDNA synthesis kit (Life
25 Technologies, Gaithersburg, MD) with an oligo dT primer incorporating a T7 RNA polymerase promoter. After second strand synthesis, the reaction mixture was extracted with phenol/chloroform, and the double-stranded DNA was isolated using a membrane filtration step (Microcon-100, Amicon). Labeled cRNA was made directly from the cDNA pool with an IVT step as described above. The total molar concentration of
30 labeled cRNA was determined from the absorbance at 260 nm and assuming an average RNA size of 1000 ribonucleotides. The convention that 1 OD is equivalent to 40 microgram of RNA, and that 1 microgram of cellular mRNA consists of 3 pmol of RNA molecules was followed. Cellular mRNA was also labeled directly without any intermediate cDNA synthesis steps. Poly (A)+ RNA was fragmented as described, and
35 the 5' ends of the fragments were kinased and then incubated overnight with a biotinylated oligonucleotide (5'-biotin-AAAAAA-3') in the presence of T4 RNA ligase (Epicentre Technologies, Madison, WI). Alternatively, mRNA has been labeled directly

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by UV-induced cross-linking to a psoralen derivative linked to biotin (Schleicher & Schuell, Keene, NH).

B. Array Hybridization and Scanning

5 The hybridization solutions contained 0.9 M NaCl, 60 mM NaH₂PO₄, 6 mM EDTA, and 0.005% Triton X-100, adjusted to pH 7.6 (referred to as 6xSSPE-T). In addition, the solutions contained 0.5 mg/ml unlabeled, degraded herring sperm DNA (Sigma, St. Louis, MO). Prior to hybridization, RNA samples were heated in the hybridization solution to 99 °C for 10 min, placed on ice for 5 min, and allowed to
10 equilibrate at room temperature before being placed in the hybridization flow cell. Following hybridization, the solutions were removed, the arrays were washed with 6xSSPE-T at 22 °C for 7 min, and then washed with 0.5 x SSPE-T at 40 °C for 15 minutes. When biotin-labeled RNA was used, the hybridized RNA was stained with a streptavidin-phycoerythrin conjugate (Molecular Probes, Eugene, OR) prior to reading.
15 Hybridized arrays were stained with 2 microgram/ml streptavidin-phycoerythrin in 6x SSPE-T at 40 °C for 5 minutes. The arrays were read using a scanning confocal microscope made for Affymetrix by Molecular Dynamics (commercially available through Affymetrix, Santa Clara, CA) The scanner uses an argon ion laser as the excitation source, with the emission detected by a photomultiplier tube through either a
20 530 nm bandpass filter (fluorescein), or a 560 nm longpass filter (phycoerythrin). Nucleic acids of either sense or antisense orientations were used in hybridization experiments. Arrays with probes for either orientation (reverse complements of each other) are made using the same set of photolithographic masks by reversing the order of the photochemical steps and incorporating the complementary nucleotide.

25

C. Quantitative Analysis of Hybridization Patterns and Intensities

Following a quantitative scan of an array, a grid is aligned to the image using the known dimensions of the array and the corner control regions as markers. The image is reduced to a simple text file containing position and intensity information using
30 software developed at Affymetrix (available with the confocal scanner). This information is merged with another text file that contains information relating physical position on the array to probe sequence and the identity of the RNA and the specific part of the RNA for which the oligonucleotide probe is designed. The quantitative analysis of the hybridization results involves a simple form of pattern recognition based on the
35 assumption that, in the presence of a specific RNA, the PM probes will hybridize more strongly on average than their MM partners. The number of instances in which the PM hybridization signal is larger than the MM signal is computed along with the average of

the logarithm of the PM/MM ratios for each probe set. These values are used to make a decision (using a predefined decision matrix) concerning the presence or absence of an RNA. To determine the quantitative RNA abundance, the average of the differences (PM minus MM) for each probe family is calculated. The advantage of the difference method is that signals from random cross-hybridization contribute equally, on average, to the PM and MM probes, while specific hybridization contributes more to the PM probes. By averaging the pairwise differences, the real signals add constructively while the contributions from cross-hybridization tend to cancel. When assessing the differences between two different RNA samples, the hybridization signals from side-by-side experiments on identically synthesized arrays are compared directly. The magnitude of the changes in the average of the difference (PM-MM) values is interpreted by comparison with the results of spiking experiments as well as the signals observed for the internal standard bacterial and phage RNAs spiked into each sample at a known amount. Data analysis programs developed at Affymetrix perform these operations automatically.

D. Oligonucleotide Probe Selection Rules

The oligonucleotide probes were selected from 600 bases of sequence at the 3' end of the translated region of each RNA. In general, probes can be selected for any useful region, and may be chosen to interrogate the 3' and 5' ends of coding regions, specific exons, or even unique untranslated regions of processed transcripts. Further selection was based on the criteria of uniqueness and hybridization characteristics. Uniqueness was assessed narrowly by comparing potential probe sequences with the full-length sequences of the other genes being monitored, and rejecting those that matched at 17 or more positions (allowing loops of up to 3 bases). A search of this type can be made more global by comparing probe sequences with the sequences of all known genes of a particular organism. Hybridization characteristics were assessed based on general sequence rules developed from the results of array hybridization experiments. Pools of specific cytokine RNAs were hybridized under stringent conditions to the 16,000 probe murine cytokine arrays. Additionally, complex RNA populations that did not contain the cytokine RNAs were hybridized to the same arrays. These two types of experiments were used to determine which probes hybridized strongly and specifically, and which ones were poor or promiscuous hybridizers. Two approaches were used to extract general rules for probe selection from these data. The first involved a direct analysis of probe behavior as a function of certain sequence features. This led to a set of heuristic rules for the selection of 20-mer probes: (1) total number of As or Ts less than 10; (2) total number of Cs or Gs less than 9; (3) number of

As or Ts in any window of 8 bases less than 7; (4) number of Cs or Gs in any window of 8 bases less than 6; (5) no more than 5 Cs or Gs in a row; (6) no more than 6 As or Ts in a row; (7) a palindrome score of less than 7 (the palindrome score is a measure of probe self-complementarity). The second approach involved the use of a neural network that was trained on the same hybridization data set used for the heuristic rules. The 20-mer probes were mapped to an 80-bit-long input vector, with the first four bits representing the base in the first position of the probe, the next four bits representing the second position, and so on. There were two outputs from the network, the hybridization intensity and the cross-hybridization intensity. The output was scaled linearly so that 95% of the outputs from the experiments fell in the range of zero to one. The network was trained using the default parameters from Neural Works Professional 2.5/NeuralWare, Pittsburgh, PA) for a backpropagation network. The neural network is a backpropagation network with 80 input neurons, one hidden layer of 20 neurons, and an output layer of 2 neurons. A sigmoid transfer function was used. As a final step in the probe selection, probes were rejected that required the greatest number of oligonucleotide synthesis steps. The maximum number of steps was limited to 60 (20 fewer than the theoretical 80 steps for the synthesis of any collection of 20-mers). This pruning step helps minimize synthesis time and cost.

20 *E. Identification of Psoriasis Markers from Skin*

In order to identify psoriasis-associated genes, the uninvolved and lesional skin from 4 psoriatic patients was analyzed using the Affymetrix hu6800 GeneChip, as described above. The uninvolved skin was used as the baseline in the analysis. 110 genes were found to have a statistically significant ($p < 0.5$) two-fold or greater increase or decrease in gene expression. The genes demonstrating a decrease in expression in psoriatic tissue as opposed to nonpsoriatic tissue are set forth in Table 1 (10 in all), whereas the genes having an increase in expression in psoriatic tissue as opposed to nonpsoriatic tissue are set forth in Table 2 (100 in all). There are several genes which were previously known to be associated with psoriasis (*e.g.*, which are differently expressed in psoriatic tissue): KRT17, S100A7, FABP5, and PI3 (Table 9). These genes were included in the analysis as a positive control, to ensure that the methodology was reliable.

The detection limit of the above assay is approximately 1:10,000 molecules. A more sensitive methodology, well-known in the art, for determining the change in expression of a gene is reverse-transcriptase polymerase chain reaction (RT-PCR). Such studies were performed on a number of genes from both uninvolved and lesional skin from several psoriasis patients. The results suggested that thirteen additional genes were

differentially expressed (either increased or decreased in expression) in psoriatic versus nonpsoriatic tissue. A GeneChip analysis was performed as above, and the results from samples taken from three different patients are set forth in Table 8. Only those genes encoding proteins expressed in at least 2 of the 3 patients are included in Table 8, and the genes are ranked in order of fold change.

F. Positional Analysis of Markers

There are several human genetic loci which are known to be correlated with psoriasis, as determined by linkage studies. These loci include 6p21.3, 17q, and 1q21 (Balendran *et al. J. Invest. Derm.* 113: 322-328, 1999; Nair *et al. Hum. Molec. Genet.* 6: 1349-1356, 1997; and Capon *et al. J. Hum. Genet.* 65: 1798-1800, 1999). The genes of the invention were mapped, and several of the marker genes are localized to these known psoriatic loci, although none of them had previously been linked to psoriasis. These genes are set forth in Tables 6 and 7 (Table 7 contains genes from a smaller, and therefore less statistically significant data set).

EXAMPLE 2: FURTHER ANALYSIS OF PSORIASIS-LINKED MARKERS

A. Identification of Psoriasis-Linked Markers from Blood

A similar GeneChip analysis to that described in Example 1(E) (above) was performed, with the exception that blood tissue, rather than skin tissue, was examined. Genes found through this analysis to increase or decrease in expression as compared to blood samples from unaffected subjects are set forth in Table 10. While several genes appear in both the skin and blood results (*e.g.*, S100A12 or SCYA4), the remainder are unique either to the blood results (Table 10) or to the skin results (Tables 1 and 2).

B. Identification of Psoriasis-Linked Markers Which Also are Markers for General Inflammation Reactions

Psoriasis is characterized by lesions and skin regions covered in whitish scale. These lesions involve, in part, a localized inflammation response. In order to ascertain whether the genes set forth in Tables 1 and 2 are differently regulated in response to psoriasis itself, or rather, in response to inflammation in general, two different assays were performed. The first assay (the 'tape strip assay') involved the stimulation of a localized inflammatory response in a subject by the application of a piece of tape to nonpsoriatic skin of a subject, followed by its rapid removal. Samples were removed

from the resulting inflamed regions of the skin, and RNA was extracted and analyzed by GeneChip as above, using normal, non-tape-contacted skin as a control.

A second assay involved the administration of an antigen, to nonpsoriatic skin of a subject in order to generate a delayed type hypersensitivity (DTH) response in the subject. As is known in the art (see, e.g., Black (1999) *Dermatology Online Journal* 5(1):7), any one of a wide variety of antigens, when injected dermally in a subject, results in a hallmark response which includes induration, swelling and monocytic infiltration into the site of the lesion within 24 to 72 hours, and which is thought to be modulated by CD4+ and CD8+ T cells. Samples were removed from the region of inflammation, and RNA was extracted and analyzed as above.

All of the genes that were previously identified that had decreased expression in psoriatic tissue as compared to nonpsoriatic tissue were found to also have decreased expression in tissue undergoing a general inflammatory reaction (Table 1). A subsection of the genes previously identified to exhibit increased expression in psoriatic tissue as compared to nonpsoriatic tissue were found to neither increase nor decrease in expression level in inflamed versus normal tissue, indicating that these genes are specific for psoriatic tissue as opposed to generally inflamed tissue (Table 3). Genes which were found to increase in expression in both psoriatic and generally inflamed tissue are set forth in Table 4.

C. *Involvement of T-Helper-1 Cells in Psoriasis*

T-helper-1 cells (TH1) are thought to play a role in the origin or pathophysiology of psoriasis. Therefore, the possibility that several of the markers of the invention may be expressed in response to T-helper cell activation was assessed.

The expression of selected markers in both activated TH1 and T-helper 2 (TH2) cells was examined.

1. *Isolation of Naïve CD4+ T Cells and Cell Culture Conditions*

Blood was obtained from the North London Blood Transfusion Service (London, UK) or from umbilical cords of neonates (Royal Free Hospital, London, UK, Chelsea and Westminster Hospital, London, UK). CD4+ T cells were purified by immunomagnetic separation using M-450 CD4 Dynabeads (Dyna, Oslo, Norway). Umbilical cord blood CD4+ cells isolated by this method were >96% CD4+ and were all of naïve phenotype.

2. Stimulation of T cells

For cytokine induction, CD4⁺ cells were preincubated overnight in IL-12 free medium and then resuspended (10⁶ cells/ml). After incubation for 0, 2, 6, and 24h in media containing 5 nM calcium ionophore ionomycin (Sigma) and 100 nM 4-phorbol-12-myristate 13-acetate (PMA) (Sigma), cells were collected by centrifugation and washed once with phosphate buffered saline prior to isolation of RNA and marker analysis as above.

As is shown in Table 5, several genes were found to have two-fold or greater expression in TH1 cells as opposed to TH2 cells. Of these, three were known to be generally induced by inflammation (MX1, GNA15, and TSSC3), while two (STAT3 and TUBB2) were not found to be inflammation-induced. Inasmuch as it is known that other disorders, such as multiple sclerosis, Crohn's disease, and rheumatoid arthritis, are known to have a TH1 component, these markers also may be correlated with these other diseases, as well as with other TH1-associated conditions.

EXAMPLE 3: ASSESSMENT OF THE EFFICACY OF rhIL-11 AND CYCLOSPORIN A TREATMENT OF PSORIASIS THROUGH MARKER ANALYSIS

One use for the markers of the invention is in the assessment of efficacy of different treatments or therapies for psoriasis or a TH1-associated condition. This example describes a marker-based analysis of the efficacy of two different treatments for psoriasis, cyclosporin A (CSA) and recombinant human IL-11 (rhIL-11), and compares the results to the histopathological analysis of the efficacy of treatment with these drugs.

A. Study Design and Subject Entry Criteria

Three subjects with extensive psoriasis (>10% body surface area affected) were treated with 2.5 or 5.0 mg/kg of rhIL-11 subcutaneously every day for 8 weeks (Trepicchio *et al.* (1999) *J. Clin. Invest.* 104:1527-1537). The dose selection of rhIL-11 was based on the outcome of a clinical trial in subjects with Crohn's disease, where biological activity was observed. In addition, 3 subjects with similar disease severity were treated with 5mg/kg per day of cyclosporin A as described previously (Gottlieb *et al.* (1992) *J. Invest. Dermatol.* 98:302-309). Upon enrollment, a psoriatic plaque was chosen for weekly assessment of lesion severity. A 6-mm punch biopsy was taken of this lesional skin before rhIL-11 or cyclosporin A treatment and at weeks 1, 4, and 8 during treatment. In addition, before initiation of treatment, a 6-mm punch biopsy was

taken from uninvolved skin at a location of the patient's choosing. Biopsies were equally divided for immunohistochemical analysis and for RNA preparation.

B. Clinical and Histopathological Assessment

5 Global clinical assessment of each subject was based on the Psoriasis Area and Severity Index (PASI) score in each subject before and during therapy as described previously (Gottlieb, *et al.* (1992) *J. Invest. Dermatol.* 98:302-309). The grading system used to measure local disease activity at the lesion biopsy site was the Psoriasis Severity Index (PSI). This index grades individual psoriasis lesions for scale, erythema, and
10 induration on a 0- to 6- point scale for each parameter (0, absent, 1, trace, 2, mild, 3, mild to moderate, 4, moderate, 5, moderate to severe, and 6, severe). The final score is the sum of the individual parameters for a range of 0-18 (Fredriksson and Pettersson (1978) *Dermatologica* 157: 238-244; and Coven *et al.* (1997) *Arch. Dermatol.* 133:1514-1522). Histopathological assessment was performed on one-half of the 6-mm
15 punch biopsy. Cryostat sections (6 micrometer) were made from frozen biopsies of psoriatic lesions obtained before and during rhIL-11 or cyclosporin A treatment. Sections were reacted with antibodies to CD3, CD8, K16, Ki67, ICAM-1, or HLA-FR and processed for immunohistochemistry as described previously. Computer-assisted image analysis was used to quantify epidermal thickness and the number of CD3+,
20 CD8+, or Ki67+ cells in tissue sections as described (Gottlieb *et al.* (1995) *Nat. Med.* 1:442-447).

C. Marker Analysis

RNA was extracted from the above biopsies, according to standard
25 methodologies, and was screened using the GeneChip assay, described above. The results are shown in Figures 1 and 2. All three patients (numbers 301, 302, and 304) were treated with 5 mg/kg CSA. As is shown in Figure 1, patient #301 had little to no change in marker expression during the course of the experiment, and similarly was found by histopathological analysis (see PSI score) to have moderate-to-severe psoriasis
30 throughout the study. Patients #302 and #304 showed a marked (greater than two-fold) decrease or increase in expression of the preponderance of the marker genes tested at the 4 week date from the initial pre-treatment sample. Similarly, the PSI score for these two individuals decreased markedly over the course of the experiment.

Equivalent findings came from the marker analysis of rhIL-11 treatment
35 (Figure 2). Patient #202 did not respond to the treatment, as assessed by PSI scores of 9-11 in this subject throughout the course of the study. The expression levels of the bulk of the markers in patient #202 throughout the study was similarly unchanged. In

contrast, the preponderance of the markers examined in two patients treated with rhIL-11 (Patients #203 and 208) were found to decrease in expression at least two-fold by week 8, and nearly all of the markers decreased in expression by week 12, as compared to the values at the beginning of the study. This result correlated well with the decrease in PSI score (to 4 and 5, respectively) seen at weeks 8 and 12.

Therefore, the expression level of the markers of the invention correlates with the efficacy of treatment of psoriasis with CSA and rhIL-11, and the efficacy of these two compounds for the treatment of psoriasis can be assessed by marker analysis.

EXAMPLE 4: ANALYSIS OF THE EXPRESSION PATTERNS OF PSORIASIS-LINKED MARKERS IN LESIONAL SKIN, NON-LESIONAL SKIN, AND INFLAMED NON-PSORIATIC SKIN

Mutations within coding regions affecting protein function or levels have traditionally been examined for disease related genes. However, mutations affecting transcriptional regulation or mRNA stability can also play a role in disease initiation or progression. This example describes the identification of such genes and/or pathways affected at the mRNA level, by performing a genome-wide scan of non-lesional and lesional psoriatic skin as well as inflamed skin from non-psoriasis patients using oligonucleotide arrays containing over 7000 human genes. A large number of differentially regulated genes were identified that distinctly classified psoriasis lesions from other skin types.

A. Study Design And Psoriasis Patient Entry Criteria

Patients with extensive plaque psoriasis (>10% body surface area affected) were considered eligible for entry into the study. Patients were treated on an outpatient basis with rhIL-11 or cyclosporin A subcutaneously every day for eight weeks. The dose and schedule selection of rhIL-11 (2.5 µg/kg/day, 5 µg/kg/day, 1 mg/once weekly and 2 mg/once weekly) and cyclosporin A (5 ug/kg/day) was as previously described (Trepicchio *et al.* (1999) *J. Clin. Invest.* 104:1527-1537). At the start of enrollment, a psoriatic plaque was chosen for weekly assessment of lesion severity scores by the study coordinator. A 6 mm punch biopsy was taken of this lesional skin prior to rhIL-11 or cyclosporin treatment and at weeks 1, 4, 8 and 12 during IL-11 or cyclosporin treatment. In addition, prior to initiation of treatment, a 6 mm punch biopsy was taken from uninvolved skin at a location of the patient's choosing. Biopsies were equally

divided for immunohistochemical analysis and for RNA preparation. Normal skin was obtained from healthy volunteers.

B. Histopathological Assessment

5 6 μ m cryostat sections were made from frozen biopsies of psoriatic lesions obtained before, during and after rhIL-11 or cyclosporin A treatment. Sections were reacted with antibodies to CD3, CD8, keratin 16, Ki67, ICAM-1, or HLA-DR and processed for immunohistochemistry as previously described (Trepicchio *et al.*, *supra*). Computer-assisted image analysis was used to quantify epidermal thickness and the
10 number of CD3+, CD8+, or Ki67+ cells in tissue sections as described. The grading system used to measure clinical disease activity was the PSI score, described above.

C. Isolation Of RNA And Preparation Of Labeled Microarray Probes

15 Total RNA was isolated from 6 mm full thickness skin biopsies using the RNeasy mini kit (Qiagen, Hilden, Germany). 2 μ g total RNA was converted to cDNA by priming with an oligo-dT primer containing a T7 RNA polymerase promoter at the 5' end. RNA was reverse transcribed with 200 units Superscript RT II at 50°C for 1 hour in 1x first-strand buffer, 10 mM DTT and 0.5 mM each dNTP (Gibco BRL, Gaithersburg, MD). Second strand cDNA was synthesized by adding 40 units DNA pol
20 I, 10 units *E. coli* DNA ligase, 2 units RNase H, 30 μ l second strand buffer, 3 ml 10 mM each dNTP, and dH₂O to 150 μ l final volume and incubating at 15°C for 2 hours. The cDNA was used as the template for in vitro transcription with a T7 RNA polymerase kit (Ambion) and incorporating biotinylated CTP and UTP (Enzo). Labeled cRNA was purified using RNeasy columns (Qiagen). RNA was concentrated
25 and the amount measured using a spectrophotometer. Labeled RNA (10 μ g) was fragmented in 40 mM Tris-acetate 8.0, 100 mM KOAc, 30 mM MgOAc for 35 minutes at 94°C in 40 μ l.

D. Hybridization To Affymetrix Microarrays And Detection Of Fluorescence

30 The labeled and fragmented RNA probes were diluted in 1x MES buffer with 100 μ g/ml herring sperm DNA and 50 μ g/ml acetylated BSA and hybridized to oligonucleotide arrays composed of 6800 human genes (Affymetrix, Santa Clara, CA). Labeled probes were denatured at 99°C for 5 minutes and then 45°C for 5 minutes and then insoluble material was removed by centrifugation. Arrays were hybridized for 16
35 hours at 45°C at 60 rpm. After hybridization, probes were removed and the cartridges washed extensively with 6 X SSPET as described by the manufacturer (Affymetrix).

Data analysis was performed using GENECHIP 3.2 software (Affymetrix) and normalizing each chip to internal bacterial gene controls. To generate the data in Figure 3 and Table 12, genes were clustered hierarchically into groups on the basis of similarity of their expression profiles by the procedure of Eisen *et al.* ((1998) *Proc.*

- 5 *Natl. Acad. Sci.* 95:14863-14868). Genes that were designated absent in all samples in a given experiment were eliminated from the analysis, as were fold changes over the designated baseline of less than 2.

E. Quantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Analysis

- 10 Quantitative RT-PCR was performed as previously described (Trepicchio *et al.*, *supra*). RNA samples from patient biopsies were treated with 10U of RQ1 DNase I (Promega) for 30 minutes at 37°C. RNA was ethanol precipitated and resuspended in diethylpyrocarbonate (DEPC)-treated sterile water. *rTth* DNA Polymerase was used to reverse transcribe and amplify 25 ng of total RNA in a single tube assay using the Perkin
15 Elmer TaqMan EZ RT-PCR kit (Perkin Elmer, Foster City, CA) with gene specific sense and antisense primers and a probe fluorescently labeled at the 5' end with 6-carboxy-fluorescein (6-FAM) using the ABI Prism 7700 sequence detection system as described by the manufacturer (Perkin Elmer). Primers and fluorescently labeled probes were generated using Primer Express software (Perkin Elmer) and were synthesized by
20 Perkin Elmer. Sequence-specific amplification was detected as an increased fluorescent signal of 6-FAM during the amplification cycle. Quantitation of gene-specific message levels was based on a comparison of the fluorescent intensity in the unknown mRNA sample to the fluorescent intensity from a standard curve of known mRNA levels. Amplification of the gene for human acidic ribosomal protein (HARP) was performed
25 on all samples tested to control for variations in RNA amounts (van Ruissen *et al.* (1998) *J. Invest. Dermatol.* 110:358-363). All genes were subsequently normalized to HARP mRNA levels. Levels of gene-specific messages were graphed as normalized message units as determined from the standard curve. A no template control was included in each amplification reaction to control for contaminating templates. For valid
30 sample analysis the fluorescent intensity in the no template control was required to be zero.

- Quantitative measures of gene expression changes were statistically evaluated using the JMP statistical discovery software package (SAS Institute, Inc., Cary, NC). An F test indicated unequal variance between groups therefore data was log
35 transformed. Differences between non-lesional to lesional skin were analyzed using 2-tailed paired T-test. An unpaired T-test was used to compare normal to uninvolved

skin. In all comparisons a p value <0.05 was used to indicate statistical significance. An ANOVA was used to compare differences across drug treatment groups.

5 *F. Patient Demographics And Global Expression Analysis Of Uninvolved, Psoriatic And Normal Skin.*

To identify disease-specific genes that were differentially regulated in psoriatic lesions, 6 mm full thickness punch biopsies were obtained from non-lesional and lesional skin of 24 patients with moderate to severe plaque psoriasis. For comparison, a limited number of biopsies from normal skin of non-psoriatic patients were obtained. 10 Biopsies were split in half with one half undergoing histological analysis and one half flash frozen and used for RNA expression analysis. Patient demographics indicated that they were evenly divided between males and females (Table 11). The average age of the psoriasis patients was 41 years with a range of 23-61 years. Patients had mean PASI scores of 29.7 with a range between 11.6 and 68. The mean biopsy site score 15 (PSI) was 9 with a range between 5 and 14 (Table 11). Histological analysis that measured epidermal thickness, Ki67+ and K16+ keratinocytes and CD3+ epidermal lymphocyte infiltration confirmed disease diagnosis (Table 11).

RNA was prepared from lesional and non-lesional skin from a subset of 8 psoriasis patients and compared to normal skin. Samples were analyzed on 20 oligonucleotide arrays containing approximately 7000 human genes (Unigem collection; National Biotechnology Information, Bethesda, MD). A global comparison of gene expression identified anywhere from 1295 to 1858 genes (19%-26% of sampled genome) expressed in non-lesional skin and 1352 to 2587 genes (19%-38% of sampled genome) expressed in lesional skin (Table 11). The number of genes expressed in 25 normal skin varied from 1383-2375 (20-22%) indicating no overt differences in global expression profiles between normal and disease.

A cluster analysis of expression patterns for all expressed genes in normal, non-lesional and lesional skin samples using a hierarchical correlation coefficient, clustered arrays of normal and non-lesional skin together and arrays of lesional skin together was 30 prepared. A dendrogram of these classifications illustrated greater inter-patient similarities in expression profiles for like skin types than intra-patient similarities between non-lesional and lesional skin types. No correlation was found between disease severity or any other available demographic in these patients to explain this clustering pattern. This result indicates that significant differences in expression 35 profiles between non-lesional and lesional skin would be observed in a differential comparison.

G. Identification Of A Psoriasis Disease Gene Classification Set

A differential mRNA expression profile of non-lesional to lesional skin from the subset of 8 patients with psoriasis was performed. Comparisons were made in a pairwise fashion between non-lesional and lesional skin from the same individual in order to reduce confounding demographic parameters. A large number of differentially regulated genes were identified. Anywhere from 340 to 1321 genes were found to increase or decrease by an average factor of 2-fold or greater within the individual patients (Table 11). Linear regression analysis revealed a correlation coefficient of 0.87 between these two populations. This degree of change was not observed in comparisons of normal to normal skin (correlation coefficient 0.97), uninvolved to uninvolved skin (correlation coefficient 0.96), and lesional to lesional skin types (correlation coefficient 0.98) between unrelated individuals. Comparison of normal skin to uninvolved psoriasis skin also indicated a high degree of similarity (correlation coefficient 0.97) with only 34 genes differing by 2-fold or greater.

To minimize the inclusion of differentially regulated genes unrelated to the disease-state, a statistical approach to the data analysis was taken. Average frequency gene expression values between non-lesional and lesional skin was calculated and a paired t-test was performed. 476 genes were identified that were statistically significantly different between these two defined groups with a confidence level of 95% or greater. This list was further refined to select only those genes whose expression levels differed on average by 2-fold or greater between non-lesional and lesional skin. 159 genes fit this second criteria (Figure 3). In contrast, 100 random permutations of the samples into two groups resulted in only 28-102 genes that were statistically significant between 2 random groups of 8 arrays, and only 6-15 genes differed by as much as 2-fold or greater between the groups. Class prediction analysis using metrics defined by Golub *et al.* ((1999) *Science* 286:531-537) indicated that this 159 gene set can be used to predict with 100% accuracy expression patterns unique to normal, non-lesional or lesional skin. These 159 genes therefore comprise a disease classification set for plaque psoriasis.

H. Characterization Of Psoriasis Classification Genes

The 159 differentially regulated genes were characterized when possible according to function. Genes involved in functions as diverse as transcriptional regulation, metabolic control, protein processing, intracellular signaling, cell cycle control, lymphocyte regulation and extracellular matrix destruction were identified (Figure 3). Many of these genes were previously reported to be differentially regulated in psoriasis such as psoriasin (S100A7), fatty acid binding protein (FABP5), elafin

(SKALP, PI3), retinoic acid binding protein (CRAB2), squamous cell carcinoma antigen 2 (SCCA2), defensin (DEFB2), keritin 17 (K17) and keritin 16 (K16) in agreement with the comparison approach used in this example (Figure 3).

Many genes not previously associated with psoriasis were also identified as being differentially regulated. For example, overexpression of multiple S100 family members such as S100A12 (calgizarin C, ENRAGE), S100A11 and S100A2, matrix metalloproteinases (MMP-12) and heparin binding protein 17 (HBP-17) were observed (Figure 3). A number of genes such as keritin 2, Apolipoprotein E (APOE), GATA3, Rb1, calponin 1 (CNN1), Cystatin 6 (CST6), TIMP-3 and TNXA were downregulated in the psoriatic lesions versus non-lesional skin. Other genes involved in inflammation and immune regulation such as the IL-4R, CD2, CD24, CD47, STAT-1, IFI27, IFI56, MX1, MnSOD, and MCP1 were elevated in lesional versus non-lesional tissue (Figure 3).

The oligo array findings for a number of differentially regulated transcripts were validated using an orthologous methodology and confirmed in a larger psoriasis patient population by quantitative PCR. Analysis of 16 additional psoriasis patients confirmed elevation of S100A12, HBP17, IL-4R, CCNF, LAD1, MAPKK3, MMP-12 and DSG3 mRNA levels in lesional skin (Figure 4A). Lower levels of CST6, TNXA, ID4, Timp-3, GATA-3, IL-5 and ApoE in lesional skin versus non-lesional skin were also confirmed (Figure 4B).

1. Comparative Expression Profiles With Other Cutaneous Inflammatory Skin Conditions

Expression profiles were generated for other cutaneous inflammatory conditions with different mechanistic components to characterize the role of these differentially regulated genes in the pathophysiology of psoriasis. These conditions included antigen-induced inflammation such as delayed type hypersensitivity reaction (DTH) and cutaneously induced skin irritation such as tape stripping. To induce a DTH reaction, 3 volunteers were sensitized with DNCB and 72 hours later challenged with a second dose of DNCB. A comparison of a biopsy obtained at the site of the DTH reaction to a noninvolved region identified from 182 to 925 genes that were differentially regulated between normal and lesional skin (Table 11). On average, 259 genes differed by 2-fold or greater in all 3 volunteers (Figure 5A). Biopsies were also obtained from normal skin and tape stripped skin from three different subjects. Anywhere from 62 to 309 genes differed in normal versus lesional skin across the 3 volunteers (Table 11). 161 genes were identified that were differentially regulated on average 2-fold or greater in all 3 tape stripped samples (Figure 5A).

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J. Mapping of Differentially Regulated Genes to Psoriasis Disease Loci

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Multiple differentially expressed genes mapped to the 6 identified psoriatic susceptibility loci (Table 12). For example, RAGE, MDF1, ID4 and TNXA mapped to the *PSOR1* loci on 6p21.3. MDF1 was overexpressed in lesional tissue and ID4, RAGE and TNXA were downregulated in lesional versus non-lesional tissue. Differential expression of several genes at the PSOR2 loci on chromosome 17q including a member of the MAP kinase family (PRKMK3; MEK3), and two genes involved in inflammatory processes, the chemokine SCYA2 and the Mac-2 binding protein, were observed.

30 Several genes that map to the PSOR4 loci on chromosome 1q21 were also
overexpressed in lesional tissue such as MTX and multiple members of the S100 gene
family such as S100A12, S100A2 and S100A9. Interestingly, S100A12 is a ligand for
the RAGE receptor that mapped to the PSOR1 loci at 6p21.3 (Hofman *et al.* (1999) *Cell*
97:889-901). One gene, ACPP, mapped to the PSOR5 loci on 3q21 and three genes
35 involved in signal transduction (CNN1, GNA15) or LDL signaling (LDLR) mapped to
the PSOR6 loci on 19p13. Quantitative RT-PCR analysis for a selected group of these
genes, such as PRKMK3, HBP17, S100A12, MTX, TNXA and ID4, in a larger patient

K. Pharmacological Treatment Of Patients Identified Pharmacogenomic Classes Of Responding Genes

15 Patients were treated with the experimental immune modulatory cytokine, rhIL-11, or with the immunosuppressant drug, Cyclosporin A. Interleukin-11 has shown activity in psoriasis patients to improve clinical and histopathological scores and these changes correlate with a reduction over time in the expression levels of type I cytokines such as IFN- γ and IL-12p40 and overexpression of type II cytokines such as IL-4 and
20 IL-5 (Trepicchio *et al.* (1999) *J. Clin. Invest.* 104:1527-1537). RhIL-11 has been shown to reduce proinflammatory cytokine production in part through the inhibition of NF- κ B nuclear translocation (Trepicchio *et al.* (1997) *J. Immunol.* 159:5661-5670).

30 RhIL-11 was administered to patients by a subcutaneous route at either a dose of 5 ug/kg/day or a single 1 mg or 2 mg dose administered once weekly. Cyclosporin A was administered at a dose of 5 µg/kg/day (Gottlieb *et al.*, *supra*). Fifteen patients were treated with rhIL-11 and 9 patients were treated with cyclosporin A for 8 weeks. Response rates to these treatments by these patients were as previously reported
35 (Gottlieb *et al.*, *supra*; Trepicchio *et al.*, *supra*). 8 out of 15 rhIL-11-treated and 8 out of 9 Cyclosporine A treated patients were considered to have responded to therapy as

defined by changes in several clinical and histopathological criteria (Trepicchio *et al.*, *supra*).

To identify genes whose expression patterns change over the course of drug treatment, comparisons in expression profiles of lesional skin were made between responding and non-responding patients prior to drug treatment and at weeks 1, 4, 8 and 12 following initiation of drug treatment. Gene chip analysis was initially performed on 4 rhIL-11-treated (3 responding and 1 non-responding) and 3 Cyclosporin-treated (2 responding and 1 non-responding) patients. The expression levels of the 165 genes that were significantly different between lesional and non-lesional skin were monitored over the course of drug treatment in these 7 patients.

Self-organizing maps (SOMs) were employed to aid in the identification of pharmacogenomic expression patterns (Tamayo *et al.* (1999) *Proc. Nat. Acad. Sci.* 96:2907-2912). Four patterns of gene expression that change over the course of drug treatment in responding patients were identified in this gene set (Figure 6). In contrast, non-responding patients saw no significant change in expression patterns of this gene set over the course of drug treatment. One of these clusters corresponded to genes whose expression levels began to return to the levels of non-lesional skin as early as one week following the initiation of drug therapy (Figure 6, Group I). These changes in expression levels preceded significant clinical improvement as measured by changes in the average PASI scores. Levels of expression of 36 genes including HBP17, SCYA2, PRKMK3, GNA15, PHB and MTX were found to change significantly in the rhIL-11-treated and/or cyclosporin A-treated responding patients at week 1 of therapy but not in the non-responding patients examined. The second pattern corresponded to genes whose levels returned to those of non-lesional skin but only at the end of drug therapy and not prior to clinical improvement (Figure 6, Group II). This cluster included 97 genes out of the 165 genes that were differentially regulated. The third expression pattern included 19 genes whose expression levels did not change over the course of therapy despite clinical improvement in the patients skin lesions (Figure 6, group III). The final cluster contained genes whose expression levels increased over the course of drug treatment. Changes in the expression levels of some of these genes such as TNXA, RAGE, ID4 and GATA-3 also proceeded clinical improvement (Figure 8, Group IV).

Changes in gene expression levels that precede clinical improvement may identify genes that play a potential causative role in disease progression or may be early markers of clinical efficacy. Quantitative RT-PCR was used to analyze gene expression changes for these early responding genes in a larger patient population. Expression levels of genes for S100A12, HBP17, K16, CCFN, SCYA2, PRKMK3, ID4, CST6 and TNXA were analyzed in 15 patients treated with rhIL-11 and 9 patients treated with

cyclosporin A (Figure 7 and data not shown). Consistent with gene chip analysis in the smaller patient population, similar changes in gene expression in response to cyclosporin A or rhIL-11 were observed as early as 1 week following drug therapy in responding patients but not in non-responding patients. In most instances, this change in gene expression preceded clinical improvement in these patients. No significant qualitative differences were observed between rhIL-11- or cyclosporin-treated patients. Following discontinuation of drug treatment at week 8, mRNA expression levels for some of these genes such as K16, S100A12 and CCNF began to rebound at week 12 towards those found in untreated lesions indicating a causative role for these genes in disease progression (Figure 7). These results also indicate that these genes or pathways defined by them may be appropriate therapeutic intervention points.

L. Gene Analysis

A comparison of gene expression profiles from psoriatic lesions and non-lesional skin from multiple patients identified over 159 differentially regulated genes. Prediction analysis indicated that these genes comprise a predictor set of the disease-state. As validation of this approach, most genes previously identified by orthologous methods as differentially regulated in psoriasis lesions such as psoriasin, elafin, FABP5, defensin, K16 and SCCA2 were identified in the gene expression profiles. Comparison of this gene set to other cutaneous inflammatory conditions, such as DTH and tape stripping, demonstrated that some of the genes that comprise this set were psoriasis-specific while others were shared in common with general skin inflammatory conditions and may play a role in other autoimmune diseases as well.

Genes activated in an antigen-specific manner during a DTH response may play a role in a variety of inflammatory conditions such as rheumatoid arthritis and Crohn's disease while genes activated during a tape-strip reaction may play a more fundamental role in generalized wounding of the skin. MMP-12, a differentially regulated gene identified in the current studies as overexpressed during the DTH response, has previously been shown through microarray analysis to be deregulated in Crohn's and RA tissue (Heller *et al.* (1997) *Proc. Natl. Acad. Sci.* 94:2150-2155). Other genes overexpressed in tape stripped samples, such as HBP17, have been shown to play a role in angiogenesis and wound healing (Czubayko *et al.* (1997) *Nat. Med.* 3:1137-1140). Finally, analysis of this gene set following patient treatment with immunomodulatory therapy indicated that pharmacogenomic analysis can be used to identify gene expression patterns that precede clinical improvement. The early responding genes may play a fundamental role in the disease process.

Based on these differential gene expression profiles, some parallels can be drawn between genes that are differentially expressed in, for example, tumor cells as well as in psoriasis lesions such as CTSB (cathepsin B), CST6 (cystatin 6), and HBP17 (heparin binding protein 17). CTSB is a lysosomal cysteine protease that plays a role in antigen processing. In the neoplastic state CTSB is overexpressed in multiple tumor types and its localization correlates with areas of angiogenesis, inflammation and necrosis (Hughes *et al.*, *supra*). It is believed to play a role in the loss of contact inhibition of tumor cells. Expression of cystatin 6, an anti-protease inhibitor of cathepsin B, is downregulated in tumor samples and this down regulation has been correlated with tumor progression (Sotiropoulou *et al.*, *supra*). Over expression of cathepsin B and down regulation of cystatin 6 in the epidermis of psoriasis patients may contribute to deregulated keratinocyte growth and differentiation. Likewise, HBP17, a heparin-binding protein for basic fibroblast growth factor (bFGF) is over-expressed in squamous cell carcinoma (Czubayko *et al.*, *supra*). Ribozyme-specific targeting of HBP17 resulted in the decreased growth and angiogenesis of xenograft tumors in mice. Interestingly, HBP17 is up regulated by p38 MAP kinase and inhibition of p38 results in a loss of HBP17 production further demonstrating a role for the p38 pathway in the progression of disease (Harris *et al.*, *supra*). A deregulated p38 MAP kinase pathway in psoriasis in addition to effects on the inflammatory process would result in up regulation of HBP17 in the epidermis resulting in increased angiogenesis and aberrant keratinocyte growth.

Interestingly, 28 genes in the psoriasis gene set identified herein map to one of the six known psoriasis-susceptibility loci. Deregulated genes that reside in chromosomal disease loci may play a fundamental role in the etiology of the disease. Autosomal or somatic mutations in regions of these 28 genes that affect transcriptional regulation or message stability can contribute to increased risk of developing psoriasis. In addition, some of these genes code for transcription factors whose aberrant expression may serve to further deregulate other down-stream pathways that further contribute to development of the disease.

These 28 genes have a variety of cellular functions ranging from transcriptional and intracellular signaling regulators to cell surface signaling receptors to secreted products involved in immune modulation and inflammation. The known functions of these genes in cell proliferation/differentiation as well as immune or inflammatory modulation support a role for the protein products of these molecules in the etiology of the disease. For example, one gene, ID4 located in the HLA locus on 6p21.3, is a dominant/negative regulator of the basic helix-loop-helix (bHLH) family of transcription factors and serve as general antagonists of cellular differentiation and proliferation in a variety of cell lineages (Reichmann *et al.*, Pagliuca *et al.*, *supra*). Cellular injury results

in a downregulation of ID4 leading to a reduction in apoptosis (Andres-Barquin *et al.* (1998), Andres-Barquin *et al.* (1999), *supra*). Reduction of ID4 expression in the epidermis, through either inherited mutations or following insult, may lead to deregulation of a number of downstream bHLH transcriptions factors that control keratinocyte growth. Another gene, S100A12 (calgranulin C) located on 1q21, is the soluble ligand for the RAGE receptor which has been implicated in activation of the proinflammatory NF- κ B pathway (Wicki *et al.*, Hofmann *et al.*, *supra*). The RAGE gene maps to the PSOR1 loci on 6p21.3. Finally, PRKMK3 is mitogen-activated protein kinase kinase 3 (MKK3), an upstream regulator of p38 MAPK (Enslin *et al.*, *supra*). p38 MAPK is activated by proinflammatory molecules such as TNF- α and plays a role in the inflammatory process. Blocking p38 activity results in a reduction in the inflammatory state (Herlaar *et al.*, *supra*).

The results described herein indicate that many members of the S100 gene family that reside in the PSOR4 loci on 1q21 are differentially regulated in a coordinate fashion in lesional tissue, consistent with previous findings (Hardas *et al.* (1996) *J. Invest. Dermatol.* 106:753-758). Mutations in a locus control region may explain these findings. A single S100 gene within the locus, such as S100A12, may contribute to risk or an unidentified gene may be in linkage disequilibrium with these mutations or multiple deregulated S100 genes in concert may contribute to risk. Changes in gene expression that precede clinical improvement may play a more causal role in disease progression as opposed to genes whose expression changes mirror clinical improvement or do not change despite clinical improvement. A subset of 36 differentially regulated genes were identified that returned to normal or uninvolved levels at time points that preceded clinical improvement following therapeutic intervention with rhIL-11 or cyclosporin A. Members of this group included genes such as ID4, HBP-17, KRT16, S100A2, S100A9, S100A12, GNA15, MTX, PRKMK3, and SCYA2 that all localized to psoriasis disease loci. In addition, a number of immune modulatory genes such as IL-4R, CD2 and GATA3 fell into this category.

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Accession	Gene Name	Category	Indication	p value	avg. uninvolved sd	uninvolved avg.	lesional sd	lesional avg.	fold change	Description
U09880_at	CRIP1	Lymphoid specific	RA, psoriasis, MS, Crohn's	0.038	36.8	20.2	13.8	8.2	0.4	Cysteine-rich heart protein (hCRHP)
U62800_at	CST6	Protein Modification and Maintenance	RA, psoriasis, MS, Crohn's	0.033	222.5	95.6	75.3	39.5	0.3	Human cystatin M (CST6) mRNA, complete cds.
X75546_at	FMOD	Protein Modification and Maintenance	RA, psoriasis, MS, Crohn's	0.016	4.3	2.2	2.3	1.9	0.5	H. sapiens mRNA for fibronectin
D84239_at	IGGFCBP	Inflammation	RA, psoriasis, MS, Crohn's	0.036	14.3	7.8	3.8	2.2	0.3	H. sapiens mRNA for IgG Fc binding protein, complete cds.
S43646_at	KRT2A	Protein Modification and Maintenance	RA, psoriasis, MS, Crohn's	0.046	286	51.1	110.3	64.9	0.4	cyokeratin 2 (human, epidermis, mRNA, 2427 nt)
U29607_at	METPEP	Protein Modification and Maintenance	RA, psoriasis, MS, Crohn's	0.015	31.3	13.4	14.3	10.1	0.5	Methionine amino peptidase
D79206_s_at	SDC4	Signal Transduction	RA, psoriasis, MS, Crohn's	0.027	40.8	14.2	22	7.6	0.5	Homosapiens gene for rydocal core protein, exon 1-5, complete cds.
J05243_at	SPTAN1	Protein Modification and Maintenance	RA, psoriasis, MS, Crohn's	0.043	10	3.2	5	1.4	0.5	Nonerythroid alpha-spectrin (SPTAN1)
D50863_at	TESK1	Transcription	RA, psoriasis, MS, Crohn's	0.03	5.3	1.7	2.8	1.7	0.5	Human mRNA for TESK1, complete cds.
AF000959_at	TMEM1	Membrane transport/ion exchange	RA, psoriasis, MS, Crohn's	0.044	5.8	3.3	2.3	1.9	0.4	Homo sapiens transmembrane protein mRNA, complete cds.

TABLE 1

Gene Name	Category	Indication	p value	are uninvolved ad uninvolved	ad lesional	ave fold change	Description
BENF	Lymphoid specific	Pontisitis	0.042	8.5	11.6	3.0	Human BENF mRNA, partial cds.
CODM	Cell metabolism	Pontisitis	0.028	15.0	33.8	2.1	Homo sapiens nuclear-encoded mitochondrial cytochrome c oxidase Va subunit mRNA, complete cds.
EFRS	Protein synthesis/processing	Pontisitis	0.046	6.3	12.0	2.8	Human translation initiation factor 5 (eIF5) mRNA, complete cds
ELP1	Protein synthesis/processing	Pontisitis	0.036	7.8	23.3	2.1	eIF-5A-eukaryotic initiation factor 5A (clone ccs 8.1) [human, placenta, Genomic, 558 nt]
HPB17	Protein Modification and Maintenance	Pontisitis	0.030	6.8	13.3	6.8	Human ELP-1 mRNA sequence.
KRT17	Protein Modification and Maintenance	Pontisitis	0.020	84.5	49.1	260.0	Human heparin binding protein (HBp17) mRNA, complete cds
LAO1	Protein Modification and Maintenance	Pontisitis	0.020	94.3	59.0	19.7	Oxykeratin 17.
LDLR	transcription	Pontisitis	0.022	8.3	23.0	8.7	Human LDL receptor mRNA, complete cds.
MDF1	Protein Modification and Maintenance	Pontisitis	0.041	10.0	4.5	29.0	Human low density lipoprotein receptor gene, exon 18
PCSK4	Protein Modification and Maintenance	Pontisitis	0.017	5.8	3.3	14.8	Human myogenic repressor 1-mf (MDRF) mRNA, complete cds
PHB	transcription	Pontisitis	0.011	3.9	25.5	8.7	Transcriptional Coactivator Pcd
RANBP2	Protein Modification and Maintenance	Pontisitis	0.048	12.3	278.0	22.5	Human RNA for RanBP2 (Ran-binding protein 1), complete cds
SPRR2A	Enzymes	Pontisitis	0.033	7.3	23.5	11.1	Small proteic (ich) protein (sprr1), clone TAN.
TOP1	Lymphoid specific	Pontisitis	0.011	10.3	4.8	23.5	Human camptothecin resistant clone CEMC2 DNA topoisomerase I mRNA, partial cds
TSSC3	cell cycle	RA, pontisitis; MS, Crohn's	0.011	7.3	22.5	8.3	IPL
BTG3	Membrane transport/ion exchange	RA, pontisitis; MS, Crohn's	0.069	11.0	3.6	29.0	Homo sapiens mRNA for tob family, complete cds
ARF6	Enzymes	RA, pontisitis; MS, Crohn's	0.046	5.3	10.5	3.7	Homo ADP-ribosylation factor (NARF6) mRNA, complete cds
ATX1A1	Membrane transport/ion exchange	RA, pontisitis; MS, Crohn's	0.024	22.3	50.3	19.3	Human copper transporter protein HAH1 (HAH1) mRNA, complete cds
CASP	Enzymes	RA, pontisitis; MS, Crohn's	0.008	3.5	21.5	5.4	Homo sapiens (clone SW93) non-gastric H+ K+-ATPase (ATP1A1) gene, exons 22, 23, complete cds.
CBR	Enzymes	RA, pontisitis; MS, Crohn's	0.050	6.8	14.5	6.1	Homo sapiens (clone SW93) non-gastric H+ K+-ATPase (ATP1A1) gene, exons 24, complete cds
CD48	Lymphoid specific	RA, pontisitis; MS, Crohn's	0.016	11.0	24.0	7.8	Human carbonyl reductase mRNA, complete cds
CDK3	cell cycle	RA, pontisitis; MS, Crohn's	0.011	4.0	8.3	1.0	Human MEN-102 glycoprotein mRNA, complete cds
CRABP2	cell metabolism	RA, pontisitis; MS, Crohn's	0.009	31.8	17.5	23.0	CD235H2/cdc25c5: homing (human, mRNA, 3118 nt)
CSNK1A1	transcription	RA, pontisitis; MS, Crohn's	0.045	6.5	11.5	6.3	Human retinoblastoma-associated protein 1 (RBAP1) gene, exons 2,4, complete cds
CSNK1E	transcription	RA, pontisitis; MS, Crohn's	0.012	8.3	3.2	9.4	Homo sapiens asen kinase I alpha locus (CSNK1A1) mRNA, complete cds
CSB	Protein Modification and Maintenance	RA, pontisitis; MS, Crohn's	0.010	74.8	172.0	6.8	Homo sapiens asen kinase I epsilon mRNA, complete cds
CYBB	Membrane transport/ion exchange	RA, pontisitis; MS, Crohn's	0.046	2.8	5.6	1.9	Human cytochrome B gene, exon 6
DLD	Cell metabolism	RA, pontisitis; MS, Crohn's	0.050	2.5	1.7	10.6	Human diacylglycerol acyltransferase b light chain p22 phospho-b-cytochrome mRNA, complete cds.
EFELL2	transcription	RA, pontisitis; MS, Crohn's	0.050	4.5	2.9	9.3	Human diacylglycerol acyltransferase gene, exon 14
EIF2A	Protein synthesis/processing	RA, pontisitis; MS, Crohn's	0.028	3.5	5.8	2.2	Human ribosomal elongation factor (eIF-2), alpha subunit mRNA, complete cds
EIF2B	Protein synthesis/processing	RA, pontisitis; MS, Crohn's	0.048	14.3	1.1	36.3	Human ribosomal elongation factor (eIF-2), alpha subunit mRNA, complete cds
E_23773	Unknown	RA, pontisitis; MS, Crohn's	0.028	5.8	1.7	12.5	Human ribosomal elongation factor (eIF-2), beta subunit (eIF-2-beta) mRNA, complete cds.
FABP5	Cell metabolism	RA, pontisitis; MS, Crohn's	0.009	48.8	167.3	3.1	Human FABP5 mRNA sequence
GIP2	Regulated by IFNg	RA, pontisitis; MS, Crohn's	0.047	4.3	1.5	64.8	Human GIP2 mRNA sequence
GARS	Protein synthesis/processing	RA, pontisitis; MS, Crohn's	0.029	5.0	47.0	17.8	Interferon-induced 17-kDa(S)-5kDa protein
GJB2	Protein Modification and Maintenance	RA, pontisitis; MS, Crohn's	0.033	5.0	2.2	61.8	Glyoxylate synthetase
GNAI5	Signal Transduction	RA, pontisitis; MS, Crohn's	0.024	9.8	50.3	31.3	Human GNAI5 mRNA sequence
GOT2	Cell metabolism	RA, pontisitis; MS, Crohn's	0.037	10.0	47.0	27.8	Human GAPDH mRNA for glyceraldehyde-3-phosphate dehydrogenase, complete cds.
GPX3	Enzymes	RA, pontisitis; MS, Crohn's	0.035	17.0	38.5	13.1	Homo sapiens mRNA for glutathione S-transferase pi (GSTP1)
GSTP1	Enzymes	RA, pontisitis; MS, Crohn's	0.011	88.0	27.5	204.0	Human GSTP1 mRNA for glutathione S-transferase pi (GSTP1)
HAL	Cell metabolism	RA, pontisitis; MS, Crohn's	0.031	5.5	2.9	51.0	Human mRNA for halodax, complete cds
HSN	Protein Modification and Maintenance	RA, pontisitis; MS, Crohn's	0.035	37.5	78.5	21.0	Human zinc binding protein (HSN) mRNA, complete cds
ID17SEP	Lymphoid specific	RA, pontisitis; MS, Crohn's	0.026	22.5	3.1	87.3	Interleukin-inducible 56 kD protein
INR5	Regulated by IFNg	RA, pontisitis; MS, Crohn's	0.010</				

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U89606_at	PYRDK	Cell metabolism	RA, psoriasis, MS, Crohn's	0.012	3.5	2.4	7.0	2.9	2.0	Human pyridoxal kinase mRNA, complete cds.
M28205_at	RAB1	Signal Transduction	RA, psoriasis, MS, Crohn's	0.046	7.0	3.8	17.8	8.0	2.5	Homo sapiens GTP-binding protein (RAB1) mRNA, complete cds.
L22942_at	RBI	Regulated by IFN-gamma	RA, psoriasis, MS, Crohn's	0.013	3.8	2.4	12.8	2.8	3.4	Human nuclear phosphoprotein mRNA, complete cds.
D38582_at	S100A11	Protein Modification and Maintenance	RA, psoriasis, MS, Crohn's	0.013	49.5	23.7	114.0	16.0	2.3	Human mRNA for calgizzarin, complete cds.
D38582_at	S100A12	Lymphoid specific	RA, psoriasis, MS, Crohn's	0.040	73.8	1.9	20.0	9.6	8.9	DNA for CAAF1 (calcium-binding protein in amniotic fluid 1)
Y07755_at	S100A2	Protein Modification and Maintenance	RA, psoriasis, MS, Crohn's	0.024	73.8	25.9	302.0	97.5	4.1	H sapiens S100A2 gene, exon 1, 2 and 3.
M87577_at	S100A7	Inflammation	RA, psoriasis, MS, Crohn's	0.044	61.0	40.8	409.0	175.7	6.7	Picorasin
M28311_x_at	S100A6	Protein Modification and Maintenance	RA, psoriasis, MS, Crohn's	0.028	17.3	7.9	396.5	182.8	23.0	Human cyclic fibrosis antigen mRNA, complete cds.
S68896_at	SCCA1	Inflammation	RA, psoriasis, MS, Crohn's	0.018	2.8	1.7	82.5	34.1	30.0	squamous cell carcinoma antigen 2 (SCCA2)
U19557_x_at	SCCA2	Protein Modification and Maintenance	RA, psoriasis, MS, Crohn's	0.010	3.8	1.0	279.5	94.8	74.5	Squamous cell carcinoma antigen 2 (SCCA2)
J04130_x_at	SCY44	Inflammation	RA, psoriasis, MS, Crohn's	0.014	2.8	1.5	6.0	1.8	2.2	Human activation (Act-2) mRNA, complete cds.
X87065_at	SEC23B	Unknown	RA, psoriasis, MS, Crohn's	0.048	4.0	2.2	8.3	3.0	2.1	H sapiens mRNA for Sec23B isoform, 2450bp.
M19869_at	SPRR1B	Protein Modification and Maintenance	RA, psoriasis, MS, Crohn's	0.028	82.3	25.5	284.0	126.4	4.7	Small proline rich protein (spr1), clone 128.
D78129_at	SOLE	Cell metabolism	RA, psoriasis, MS, Crohn's	0.040	8.0	1.8	40.0	18.7	5.0	Human adult (34 year old) Male liver mRNA for squalene epoxidase, partial cds.
M97935_x_at	STAT1	Regulated by IFN-gamma	RA, psoriasis, MS, Crohn's	0.037	9.3	3.9	30.5	15.1	3.3	Homo sapiens transcription factor ISGF-3 mRNA, complete cds.
L23277_at	STAT3	Transcription	RA, psoriasis, MS, Crohn's	0.042	7.8	2.8	19.3	6.7	2.5	Homo sapiens DNA-binding protein (APRF) mRNA, complete cds.
T82_1	T82_1	Protein synthesis/processing	RA, psoriasis, MS, Crohn's	0.040	4.8	3.0	10.5	3.1	2.2	Human TB3-1 mRNA, complete cds.
M88447_maf_at	TGM1	Protein synthesis/processing	RA, psoriasis, MS, Crohn's	0.014	8.5	3.9	53.8	16.2	6.3	H sapiens keratinocyte transglutaminase gene, complete cds.
L10386_at	TGM3	Protein synthesis/processing	RA, psoriasis, MS, Crohn's	0.011	8.3	3.8	61.8	19.3	7.5	Homo sapiens transglutaminase E3 (TGASE3) mRNA, complete cds.
HG22759-AT7235_at	TP11	Lymphoid specific	RA, psoriasis, MS, Crohn's	0.029	49.0	20.4	96.3	31.1	2.0	Triosephosphate isomerase
U82275-AT7235_at	TP11	Protein Modification and Maintenance	RA, psoriasis, MS, Crohn's	0.045	28.5	8.3	85.5	38.1	3.0	Tubulin, Beta
M61670_at	TUBB2	Unknown	RA, psoriasis, MS, Crohn's	0.024	4.0	2.9	12.3	4.6	3.1	Ubiquitin carrier protein (E2-EPP)
U73379_at	UBE2F	cell cycle	RA, psoriasis, MS, Crohn's	0.028	9.3	1.0	22.3	7.0	2.4	Human cyclin-selective ubiquitin carrier protein (UOCRF51) gene, exon 2.
L32977_at	UOCRF51	Cell metabolism	RA, psoriasis, MS, Crohn's	0.039	22.0	7.8	48.0	17.8	2.1	Homo sapiens (clone 117252) ubiquitin cytochrome c reductase Rhesia (non-sulphur protein (UOCRF51) gene, exon 2.

TABLE 2 (continued)

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Accession	Gene Name	Category	Indication	p value	ave uninvolved	sd uninvolved	ave lesioned	sd lesioned	ave fold change	Description
U17077_at	BENE	Lymphoid specific	psoriasis	0.042	8.5	4.0	25.8	11.6	3.0	Human BENE mRNA, partial cds.
M27760_at	COX5A	Cell metabolism	psoriasis	0.029	16.0	6.1	33.8	13.2	2.1	Homo sapiens nuclear-encoded mitochondrial cytochrome c oxidase Va subunit mRNA, complete cds.
U49436_at	EIF5	Protein synthesis/processing	psoriasis	0.033	4.3	2.6	12.0	5.0	2.8	Human translation initiation factor 5 (eIF5) mRNA, complete cds.
S72024_s_at	EIF5A	Protein synthesis/processing	psoriasis	0.046	6.3	2.8	13.3	6.7	2.1	eIF-5A=autolytic initiation factor 5A (clone cos 9.1) [human, placenta, Genomic, 558 nt].
M68458_at	ELP1	Protein synthesis/processing	psoriasis	0.036	7.8	3.2	23.3	11.6	3.0	Human ELP-1 mRNA sequence.
M69047_at	HBP17	Protein Modification and Maintenance	psoriasis	0.037	8.8	5.1	59.5	29.8	6.8	Human heparin binding protein (HBP17) mRNA, complete cds.
Z19374_ma1_at	KRT17	Protein Modification and Maintenance	psoriasis	0.020	64.5	49.1	260.0	72.2	4.0	cyokeratin 17.
U42406_at	LAD1	Protein Modification and Maintenance	psoriasis	0.029	26.3	10.4	59.0	19.7	2.2	Human ladinin (LAD) mRNA, complete cds.
L00352_at	LDLR	Protein Modification and Maintenance	psoriasis	0.030	8.3	3.4	23.0	8.2	2.8	Human low density lipoprotein receptor gene, exon 18.
U78313_at	MDF1	Transcription	psoriasis	0.022	3.3	1.3	7.0	2.4	2.2	Human myogenic repressor 1-nt (MDF1) mRNA, complete cds.
HG4297-IT4567_at	PCSK4	Protein Modification and Maintenance	psoriasis	0.041	10.0	4.5	29.0	10.8	2.9	Transcriptional Coactivator Pcd
S85855_at	PHB	Protein Modification and Maintenance	psoriasis	0.017	5.8	3.3	14.8	5.7	2.6	prohibitin [human, mRNA, 1043 nt].
D58075_at	RANBP1	transcription	psoriasis	0.011	8.3	3.9	25.5	8.7	3.1	Human mRNA for RanBP1 (Ran-binding protein 1), complete cds.
M21302_at	SPRR2A	Protein Modification and Maintenance	psoriasis	0.048	12.3	3.3	276.0	139.7	22.5	Small proline rich protein (sprt), clone 174N.
U07806_s_at	TOP1	Enzymes	psoriasis	0.033	7.3	4.6	23.5	11.1	3.2	Human camptothecin resistant clone CEMC2 DNA topoisomerase I mRNA, partial cds.

TABLE 3

TABLE 4

Accession	Gene Name	Category	Indication	p value	are uninvolved	sd uninvolved	are involved	sd involved	are fold change	Description
AF01294_at	TS5C3	Lymphoid specific	RA, psoriasis, MS, Crohn's	0.009	10.5	4.7	28.5	8.3	2.8	IPL
D64110_at	TS5C3	cell cycle	RA, psoriasis, MS, Crohn's	0.009	11.0	3.6	22.0	6.5	2.0	Homo sapiens mRNA for tob family, complete cds.
M87762_at	ARF5	Membrane transport/exchange	RA, psoriasis, MS, Crohn's	0.046	5.3	1.5	10.5	3.7	2.0	Human ADP-ribosylation factor (ARF5) mRNA, complete cds.
U74660_at	ATOX1	Enzymes	RA, psoriasis, MS, Crohn's	0.024	22.3	10.0	50.3	19.3	2.3	Human copper transport protein (HAH1) mRNA, complete cds.
U74660_at	ATP1A1	Membrane transport/exchange	RA, psoriasis, MS, Crohn's	0.009	3.5	2.4	21.5	5.4	6.1	Homo sapiens (clone ISW39) non-gastric H ⁺ -K ⁺ -ATPase (ATP1A1) gene, exons 22, 23, complete cds.
D25866_at	ATP1A1	Protein synthesis/processing	RA, psoriasis, MS, Crohn's	0.001	6.8	4.0	14.5	4.0	2.1	Human mRNA for cysteine protease, complete cds.
J40595_at	CBR	Enzymes	RA, psoriasis, MS, Crohn's	0.050	11.0	2.2	23.3	7.8	2.1	Human carbonyl reductase mRNA, complete cds.
M37766_at	CD48	Lymphoid specific	RA, psoriasis, MS, Crohn's	0.016	4.0	2.2	8.3	1.0	2.1	Human MEM-102 glycoprotein mRNA, complete cds.
CD228	CD228	cell cycle	RA, psoriasis, MS, Crohn's	0.038	7.0	3.6	15.8	6.3	2.3	CDC25H2/cdc25+ homolog (human, mRNA, 3118 nt).
CDKN3	CDKN3	cell cycle	RA, psoriasis, MS, Crohn's	0.031	3.8	1.7	7.8	1.3	2.1	Homo sapiens protein tyrosine phosphatase (CIP2) mRNA, complete cds.
M87815_at	CRABP2	Cell metabolism	RA, psoriasis, MS, Crohn's	0.006	31.8	7.5	123.0	24.1	3.9	Human retinoic acid-binding protein II (CRABP-II) gene, exon 2-4, complete cds.
L37042_at	CNSK1A	transcription	RA, psoriasis, MS, Crohn's	0.045	6.5	3.0	21.5	9.4	3.3	Homo sapiens casein kinase I alpha isoform (CNSK1A) mRNA, complete cds.
U74660_at	CNSK1E	transcription	RA, psoriasis, MS, Crohn's	0.012	6.3	4.6	12.3	3.2	2.0	Homo sapiens casein kinase I epsilon mRNA, complete cds.
U66892_m1	CSTB	Protein Modification and Maintenance	RA, psoriasis, MS, Crohn's	0.010	74.8	33.5	172.0	64.8	2.3	Human cytochrome b561 gene, complete cds.
U29463_s_at	CYB5A	Membrane transport/exchange	RA, psoriasis, MS, Crohn's	0.046	2.8	1.7	5.8	1.9	2.1	Human cytochrome b561 gene, exon 6.
M21186_at	DLF	Enzymes	RA, psoriasis, MS, Crohn's	0.050	2.5	1.7	10.8	6.4	4.3	Human neurotrophin cytochrome b light chain p22 phagocyte b-cytochrome mRNA, complete cds.
U86629_at	FELL2	transcription	RA, psoriasis, MS, Crohn's	0.028	4.5	2.9	9.3	3.1	2.1	Human RFA polymerase II elongation factor ELL2, complete cds.
J02645_at	EIF2A	Protein synthesis/processing	RA, psoriasis, MS, Crohn's	0.022	8.3	3.0	19.8	7.7	2.4	Human RFA polymerase II elongation factor ELL2, complete cds.
EIF2B	EIF2B	Protein synthesis/processing	RA, psoriasis, MS, Crohn's	0.048	14.3	6.1	36.3	14.1	2.5	Human RFA polymerase II elongation factor ELL2, complete cds.
U90904_at	E23773	Unknown	RA, psoriasis, MS, Crohn's	0.026	5.8	1.7	12.5	3.1	2.2	Human RFA polymerase II elongation factor ELL2, complete cds.
M94566_at	FABP5	Cell metabolism	RA, psoriasis, MS, Crohn's	0.009	48.8	8.6	167.3	38.9	3.4	Human RFA polymerase II elongation factor ELL2, complete cds.
M13755_at	G1P2	Regulated by IFN-gamma	RA, psoriasis, MS, Crohn's	0.047	4.3	1.5	84.8	48.6	19.9	Human RFA polymerase II elongation factor ELL2, complete cds.
GARS	GARS	Protein synthesis/processing	RA, psoriasis, MS, Crohn's	0.029	17.5	5.2	47.0	17.8	2.7	Human RFA polymerase II elongation factor ELL2, complete cds.
G1B2	G1B2	Protein Modification and Maintenance	RA, psoriasis, MS, Crohn's	0.033	37.5	27.5	204.0	46.2	2.3	Human RFA polymerase II elongation factor ELL2, complete cds.
M83904_at	G22532	Signal Transduction	RA, psoriasis, MS, Crohn's	0.031	5.5	2.9	51.0	22.2	9.3	Human RFA polymerase II elongation factor ELL2, complete cds.
G072	G072	Cell metabolism	RA, psoriasis, MS, Crohn's	0.037	10.0	4.7	27.8	11.9	2.8	Human RFA polymerase II elongation factor ELL2, complete cds.
GPX3	GPX3	Enzymes	RA, psoriasis, MS, Crohn's	0.035	17.0	13.0	36.5	13.1	2.1	Human RFA polymerase II elongation factor ELL2, complete cds.
GSTP1	GSTP1	Enzymes	RA, psoriasis, MS, Crohn's	0.011	88.0	2.0	87.3	33.9	3.9	Human RFA polymerase II elongation factor ELL2, complete cds.
M24445_s_at	HSL	Cell metabolism	RA, psoriasis, MS, Crohn's	0.031	5.5	2.9	51.0	22.2	9.3	Human RFA polymerase II elongation factor ELL2, complete cds.
U10626_at	HSL	Cell metabolism	RA, psoriasis, MS, Crohn's	0.031	5.5	2.9	51.0	22.2	9.3	Human RFA polymerase II elongation factor ELL2, complete cds.
U30357_at	HSN	Protein Modification and Maintenance	RA, psoriasis, MS, Crohn's	0.035	37.5	27.5	204.0	46.2	2.3	Human RFA polymerase II elongation factor ELL2, complete cds.
HG3342-HT3519	IFIT2P5P	Lymphoid specific	RA, psoriasis, MS, Crohn's	0.029	22.5	3.1	87.3	33.9	3.9	Human RFA polymerase II elongation factor ELL2, complete cds.
J04184_at	IFIT2P5P	Regulated by IFN-gamma	RA, psoriasis, MS, Crohn's	0.010	49.8	17.9	148.0			

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M07955_s_at	STAT1	Regulated by IFN γ	RA, psoriasis, MS, Crohn's	0.037	9.3	3.9	30.5	15.1	3.3	Homo sapiens transcription factor ISGF-3 mRNA, complete cds.
L29277_at	STAT3	transcription	RA, psoriasis, MS, Crohn's	0.042	7.8	2.8	19.3	6.7	2.5	Homo sapiens DNA-binding protein (APRF) mRNA, complete cds.
M73715_s_at	TB3_1	Protein synthesis/processing	RA, psoriasis, MS, Crohn's	0.040	4.8	3.0	10.5	3.1	2.2	Human TB3-1 mRNA, complete cds.
M28447_m1_at	TGM1	Protein synthesis/processing	RA, psoriasis, MS, Crohn's	0.014	8.5	3.9	53.8	16.2	6.3	Hisapiens keratinocyte transglutaminase gene, complete cds.
L10386_at	TGM3	Protein synthesis/processing	RA, psoriasis, MS, Crohn's	0.011	8.3	3.8	61.8	19.3	7.5	Homo sapiens transglutaminase E3 (TGASE3) mRNA, complete cds.
R02279-IT2375_7911		Lymphoid specific	RA, psoriasis, MS, Crohn's	0.029	48.0	20.4	96.3	31.1	2.0	Triosephosphate isomerase
R04322-IT14592_TUBB2		Protein Modification and Maintenance	RA, psoriasis, MS, Crohn's	0.045	28.5	8.3	85.5	38.1	3.0	Tubulin, Beta
M61670_at	UBE2F	Ubiquitin	RA, psoriasis, MS, Crohn's	0.024	4.0	2.9	12.3	4.6	3.1	Ubiquitin carrier protein (E2-EPPF)
U73379_at	UBCRF1	cell cycle	RA, psoriasis, MS, Crohn's	0.028	9.3	1.0	22.3	7.0	2.4	Human cyclin-selective ubiquitin carrier protein mRNA, complete cds.
L32977_at	UBCRF1	Cell metabolism	RA, psoriasis, MS, Crohn's	0.039	22.0	7.6	46.0	17.8	2.1	Homo sapiens (clone ft7252) ubiquitin cytochrome c reductase Rieske iron-sulphur protein (UQCRCF1) gene, exon 2.

TABLE 4 (continued)

TABLE 5

Gene Name	Freq Th2	Freq Th1	Fold-change	Known Psor/Skin Gene	Known Psoriatic Loci	Known DTH Gene	Known Tape Strip Gene	Gene Category	Gene Description
STAT3	22	44	2.0		Yes			Transcription	DNA-binding protein (APRF)
MX1	2	18	7.9			Yes	Yes	Regulated by IFNg	p78 protein
GNA15	14	32	2.3			Yes	Yes	Signal Transduction	G-alpha 16 protein
TSSC3	11	32	2.9				Yes	Lymphoid specific	IPL
TUBB2	4	17	4.2					Protein Modification and Maintenance	Beta Tubulin

Markers of the Invention Which Map to Known Psoriatic Loci

Psoriatic Loci	Chromosome	Gene	Category	Gene Description
I	6p21.3	MDF1	Transcription	Myogenic repressor 1-mf (MDF1)
II	17q	CYB561	Membrane transport/ Ion exchange	Cytochrome b561
		MAP2K3	Signal Transduction	MAP kinase kinase 3b
		KRT17	Protein Modification and Maintenance	Cytokeratin 17
		PHB	Protein Modification and Maintenance	Prohibitin
		SCYA4	Inflammation	Activation (Act-2)
		STAT3	Signal Transduction	DNA-binding protein (APRF)
		LGALS3BP	Inflammation	Mac-2 binding protein
		P4HB	Protein Modification and Maintenance	Thyroid hormone binding protein (p55)
IV	1q21	S100A9	Inflammation	Cystic fibrosis antigen
		S100A11	Protein Modification and Maintenance	Calgizzarin
		S100A2	Protein Modification and Maintenance	S100 calcium-binding protein A2
		S100A7	Protein Modification and Maintenance	S100 calcium-binding protein A7
		S100A12	Protein Modification and Maintenance	CAAF1 (calcium-binding protein in amniotic fluid 1)
		SPRR2A	Protein Modification and Maintenance	Small proline rich protein (sprl)
		SPRR1B	Protein Modification and Maintenance	Small proline rich protein (sprl)
		CRABP2	Cell metabolism	Retinoic acid-binding protein II (CRABP-II)
		CD48	Lymphoid specific	MEM-102 glycoprotein

TABLE 6

Psoriatic Loci	Chromosome	Gene	Gene Description
I	6p21.3	ID4	Dominant/negative inhibitor of helix-loop-helix transcription factors
		PBX2	Pre-B-cell leukemia transcription factor 2 Homeobox transcription factor
		RAGE	Receptor for advanced glycosylation end products (i.e. S100A12) In HLA class III region containing NOTCH4 gene

TABLE 7

NAME	Intensity. P/A. psoriasis	P/A. Exp.	Intensity Non-lesional	P/A. Bas.	Diff. Fold	FUNCTION
Defensin	6820	P	2	A	I	341 Antibacterial agent. Expressed in the skin and respiratory tract. Belongs to the beta-defensin family
PD-ECGF	1520	P	0	A	I	76 Platelet-derived endothelial cell growth factor. Angiogenic factor
TPA	652	P	-34	A	I	32.6 Tissue plasminogen activator. Active in tissue remodeling and destruction.
MMP-12	487	P	-35	A	I	24.3 Metalloproteinase, macrophage elastase. Secreted by alveolar macrophages
S100A8	344	P	-2	A	I	17.2 Expressed in macrophages during chronic inflammation
S100A2	170	P	10	A	I	8.5 May act as a modulator against excess calcium accumulation. A subset of epithelial cells and keratinocytes.
IFL-27	100	P	0	A	I	5.0 Interferon-inducible protein-27
MMP-16	84	P	154	-I	I	4.2 Metalloproteinase, gelatinase
MMP-2	239	P	64	A	I	4.0 Metalloproteinase; gelatinase, collagenase
TIMP-3	40	A	90	P	D	2.0 Tissue inhibitor of metalloproteinases
APO-E	1040	P	5578	P	D	5.6 Apolipoprotein E. Mediates binding and catabolism of lipoprotein particles.
ID4	26	M	200	P	D	7.7 Dominant/Negative inhibitor of helix-loop-helix transcription factors. Chr. Location: PSORS1 loci (6p21.3)
PBX2	11	A	425	P	D	20 Pre-B cell leukemia transcription factor. Homeobox family member. Chr. Location: PSORS1 Loci (6p21.3)

TABLE 8: Comparison of uninvolved and psoriatic skin samples from 3 different psoriasis patients using 6800 human genechip. Only genes encoding proteins expressed in at least 2 of 3 patients are included and genes are ranked in order of fold change.

Genes Previously Implicated in Psoriasis

Known Genes	Gene Name	Maps to Psoriatic Loci	Gene Category	Gene Description
Psoriatic	KRT17	Yes	Protein Modification and Maintenance	cytokeratin 17
	S100A7	Yes	Protein Modification and Maintenance	S100 calcium-binding protein A7
	FABP5	-	Cell metabolism	Fatty acid binding protein
	PI3	-	Protein Modification and Maintenance	Elafin, skin-derived (SKALP)

TABLE 9

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name	p values	ave normal freq	ave psoriatic freq	fold change	in psor skin	Gene ID	
GOS2	0.01879	1.4	18.16666667	12.97619048	-	Human GOS2 gene, 5' flank and cds.	
IL8	0.05153	1.8	16.66666667	9.259259259	-	Human interleukin 8 (IL8) gene, complete cds.	
IL1B	0.02254	1.6	14.33333333	8.958333333	-	Human gene for prointerleukin 1 beta.	
PROTEIN	0.00028	13.8	80	5.797101449	-	Human cellular oncogene c-fos (complete sequence).	
PTGS2	0.00668	1.3	7.5	5.769230769	-	Human cyclooxygenase-2 (hCox-2) gene, complete cds.	
IEX1	0.0293	2.5	14.33333333	5.733333333	-	IEX-1=radiation-inducible immediate-early gene [human, placenta, mRNA Partial, 1223 nt].	
HLX1	0.00093	1.1	4.666666667	4.242424242	-	Human homeobox gene, complete cds.	
HMR	0.007	2	8.333333333	4.166666667	-	Human TR3 orphan receptor mRNA, complete cds.	
ZFP36	0.00099	15.7	63.33333333	4.033970276	-	H sapiens zinc finger transcriptional regulator mRNA, complete cds.	
DUSP2	0.01329	1.8	6.666666667	3.703703704	-	Homo sapiens protein tyrosine phosphatase (PAC-1) mRNA, complete cds.	
GRO2	0.02396	1.1	4	3.636363636	-	Human gro-beta mRNA, complete cds.	
RGS2	0.00064	16.8	58.33333333	3.472222222	-	Human helix-loop-helix basic phosphoprotein (GOS8) gene, complete cds.	
K246_NOTCH3	0.01717	2.2	7	3.181818182	-	Human mRNA for KIAA0246 gene, partial cds.	
ETR101	0.00188	26.6	83.33333333	3.13283208	-	Human transcription factor ETR101 mRNA, complete cds.	
BTG2	0.00304	14.6	45.33333333	3.105022831	-	Human BTG2 (BTG2) mRNA, complete cds.	
SLC2A3	0.00031	7.5	23	3.066666667	-	Human glucose transporter-like protein-III (GLUT3), complete cds.	
C5R1	0.00033	8.3	24.83333333	2.991967871	-	Human C5a anaphylatoxin receptor mRNA, complete cds.	
TNFR1	0.03069	4.5	12.83333333	2.851851852	-	Homo sapiens tumor necrosis factor receptor mRNA, complete cds.	
FCGR1A	0.01287	3.3	9.333333333	2.828282828	-	Human IgG Fc receptor I gene, exon 6 and complete cds.	
ANPEP	0.05804	4.4	12.16666667	2.765151515	-	Human aminopeptidase N/CD13 mRNA encoding aminopeptidase N, complete cds.	
FOSB	0.01048	3.8	10.33333333	2.719298246	-	Human GOS3 mRNA, complete cds.	
ARHB	0.00255	1.9	5.166666667	2.719298246	-	Human ras-related rho mRNA (clone 6), partial cds.	
EGR2	0.05985	1.2	3.166666667	2.638888889	-	Human early growth response 2 protein (EGR2) mRNA, complete cds.	
PTGS2	0.00989	1.1	2.833333333	2.575757576	-	Human PTGS2 gene for prostaglandin endoperoxide synthase-2, complete cds.	
SCYA4	0.01251	2.2	5.666666667	2.575757576	-	Human cytokine (SCYA2) gene, exon 3.	
PF4V1	0.04528	2.6	6.666666667	2.564102564	-	Human platelet factor 4 variation 1 (PF4var1) gene, complete cds.	
MAD3	0.02147	24.2	62	2.561983471	-	Homo sapiens MAD-3 mRNA encoding IkB-like activity, complete cds.	
BCL6	0.01111	2.7	6.833333333	2.530864198	-	Human zinc-finger protein (bcl-6) mRNA, complete cds.	
CMKBR1	0.0376	1.4	3.5	2.5	-	Human mRNA for HM145.	
EZF	0.02299	1.6	4	2.5	-	Human zinc finger transcription factor hEZF (EZF) mRNA, complete cds.	
TUBA1	0.00205	11.6	28.66666667	2.471264368	-	Human gene for alpha-tubulin (b alpha 1).	
SCYA4	0.05538	11.1	27.33333333	2.462462462	SCYA4	Human activation (Act-2) mRNA, complete cds.	
SAT	0.00096	8.6	21.16666667	2.46124031	-	Human spermidine/spermine N1-acetyltransferase (SSAT) gene, complete cds.	
PLSCR1	0.00285	3.4	7.833333333	2.303921569	PLSCR1	Homo sapiens phospholipid scramblase mRNA, complete cds.	
OC116	0.03589	5.1	11.66666667	2.287581699	-	Human specific 116-kDa vacuolar proton pump subunit (OC-116kDa) mRNA, complete cds.	
CALR	0.00687	3.6	8.166666667	2.268518519	-	Human autoantigen calreticulin mRNA, complete cds.	
A1P	0.00062	5	11.33333333	2.266666667	-	Human A1 protein mRNA, complete cds.	
TETTRL	0.0431	4.9	11	2.244897959	-	Human tetracycline transporter-like protein mRNA, complete cds.	
SPARC	0.00934	19.4	42.66666667	2.199312715	-	Human SPARC/osteonectin mRNA, complete cds.	
DNM2	0.03113	3.8	8.166666667	2.149122807	-	Homo sapiens dynamin (DNM) mRNA, complete cds.	
251_COSC14H10	0.01004	2.6	5.5	2.115384615	-	Human mRNA for KIAA0251 gene, partial cds.	

TABLE 10

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ALDH7	0.00102	5.7	12	2.105263158 -	Human aldehyde dehydrogenase ALDH7 mRNA, complete cds.
CSRP1	0.00129	4	8.333333333	2.083333333 -	Human cysteine-rich protein (CRP) gene, exons 5 and 6.
CSF3R	0.0028	9.8	20.33333333	2.074829932 -	Human granulocyte colony-stimulating factor receptor (CSF3R) mRNA, complete cds.
TOP1	0.00626	5.5	11.16666667	2.03030303 TOP1	Human camptothecin resistant clone CEM/C2 DNA topoisomerase I mRNA, partial cds.
RAB31	0.05361	5.8	11.66666667	2.011494253 -	Human low-Mr GTP-binding protein (RAB31) mRNA, complete cds.
S100A12	0.01998	17.6	35.33333333	2.007575758 S100A12	Human DNA for CAAF1 (calcium-binding protein in amniotic fluid 1), complete cds.
OCLSF	0.01305	4	8	2 -	Human osteoclast stimulating factor mRNA, complete cds.
NF2TS	0.0055	30.9	17.16666667	0.5555555556 -	Neurofibromatosis 2 Tumor Suppressor (Gb.L27065)

TABLE 10 (continued)

Demographic and clinical characteristics of psoriasis patients and controls

Clinical Scores

Patient #	Sex/Age	Race	Treatment	Skin Type	Biopsy Site	Clinical Scores			epidermal thickness	Ki67+ keratinocytes	Histological Data		Gene Chip Data	
						PASI	PSI				epidermal CD3+	K16+	present genes	differentially expressed genes
201	F/47	W	rhIL-11	nonlesion	back								1295	
202	M/31	W	rhIL-11	lesion	back	20.7	5						1352	519
203	M/53	W	rhIL-11	nonlesion	back	14.8	11						1428	
204	M/50	W	rhIL-11	lesion	rt thigh	19.8	10						2059	878
205	M/44	W	rhIL-11	nonlesion	back	34.8	7						1671	416
206	F/23	W	rhIL-11	lesion	back	11.6	10						1653	
207	F/		rhIL-11	nonlesion	back	24.6	7						2587	1321
208	M/36	W	rhIL-11	lesion		13.4	6							
210	M/61	W	rhIL-11	nonlesion		22.6	7							
211	F/45		rhIL-11	lesion	buttocks	13.5	9						1776	
212	F/61	W	rhIL-11	nonlesion	back	42.2	10						1868	543
213	F/49	W	rhIL-11	lesion	buttocks	19.8	9							
221	M/57	W	rhIL-11	nonlesion	buttocks	19.6	9							
222	F/52	W	rhIL-11	lesion	back	12.4	8						1743	340
223	F/32	W	rhIL-11	nonlesion	back	21.8	9						1812	
301	M/53	W	cyclosporin A	lesion	back	11.7	9						1858	454
302	F/47	W	cyclosporin A	nonlesion		68	9						2027	
303	F/46	W	cyclosporin A	lesion		29	8							
304	F/	B	cyclosporin A	nonlesion		20	7							
305	M/		cyclosporin A	lesion		37.8	9						1680	349
310	M/51	W	cyclosporin A	nonlesion		33.6	7						1773	
				lesion		44.1	12							

TABLE 11

311	F/51	cyclosporin A	nonlesion				
312	F/47	cyclosporin A	lesion	66	14		
313	F/	cyclosporin A	nonlesion	66	12		
401	Mayerson	tape strip	nonlesion	63	11		
402	Collins	tape strip	lesion			1383	309
403	F/ Hoffman	tape strip	normal			1619	
404	Komar	tape strip	lesion			1534	62
501	Salcedo	DTH	normal			1683	
503	J. McCormack	DTH	lesion			1502	283
504	Kapadia	DTH	nonlesion			1724	
			lesion	12.1		2187	
			nonlesion			2336	492
			lesion			2375	
			normal			2514	925
			lesion			1612	
			normal			2331	182
			lesion				

TABLE 11 (continued)

